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JOURNAL OF DAIRY SCIENCE

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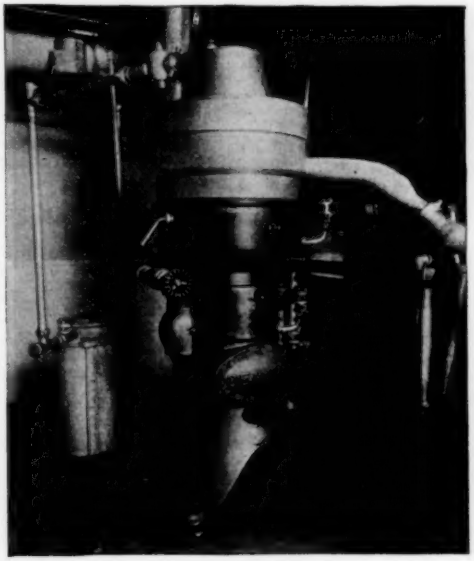

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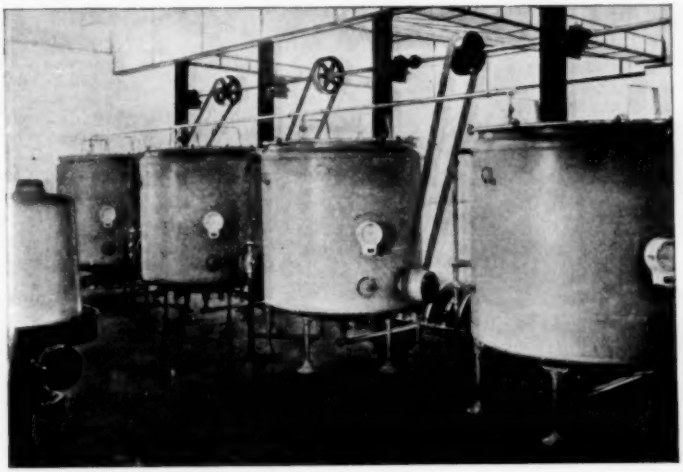

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Among his conclusions the author says:

"It is evident that *utensils* are the *major factor* to be considered in the production of clean milk of maximum keeping qualities."

"Adequate steam treatment, or where this is impracticable, chlorination, is most essential in the care of utensils."

"Next to utensils, is the necessity for thorough cleaning of *flanks* and *udders* of the *cows.*"

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A METHOD FOR THE SAPONIFICATION OF BUTTER FAT FOR DETERMINING THE REICHERT MEISSL NUMBER*

GEORGE SPITZER AND W. F. EPPLE

Dairy Department, Purdue University Agricultural Experiment Station, LaFayette, Indiana

In this laboratory for the past fifteen years many thousand Reichert-Meissl determinations have been made. During this period we have used, at different times, one or the other official methods. After numerous trials we confined our work to the saponification of the fat under pressure with alcohol, preferring this method to the other two methods. In spite of its apparent simplicity, it frequently happened that the alcohol was not completely removed, due to the solution and mixture with the soap formed during saponification.

We, therefore, tried to saponify the fat without the use of alcohol. After numerous trials we found that by adding concentrated solution of potassium hydrate directly to the 5 grams of fat, the fat being previously heated to 80° to 100°C., shaking slightly to thoroughly mix the fat and potassium hydrate solution. This mixture was placed in an electric oven for thirty to forty minutes. At the end of this time saponification was complete. The results obtained by this method of saponification were checked against the three official methods with very consistent results.

METHOD

Use a 250-cc. low form Griffin beaker, pyrex glass preferred. Balance beaker, place a 5-gram weight on opposite pan and with a pipette run into beaker approximately 5 grams of melted butter fat, weigh added fat to the third decimal place.

Place the weighed fat in an oven heated to 100°C. until temperature of fat reaches 80° to 100°C., remove and add 3 cc. of

* Received for publication January 14, 1927.

potassium hydrate solution,[1] by a circular motion mix fat and potash solution. After being mixed in this manner incline beaker to an angle of 45° and rotate. Some of the mixture will spread on side of beaker. Continue this rotation until the mixture thickens by cooling. Place beaker in oven at 100°C. and let it remain for thirty to forty-five minutes to complete saponification. Place beaker in desiccator to cool also to prevent the absorption of CO_2.

To transfer the saponified fat to the distilling flask break the cake of soap into small pieces by means of a glass rod, transfer the broken pieces to flask, the adhering particles on beaker and glass rod are removed by means of previously boiled hot distilled water. This water must be accurately measured. The total volume added should be 130 cc. When soap is dissolved in the distilling flask add 10 cc. of dilute sulphuric acid A. O. A. C. P. 247—23 (d), then proceed as directed by the official method, p. 248—25, 1920.

ADVANTAGES OF THIS METHOD

1. Speed in weighing the fat, weighing is made directly by pouring the fat into the counterpoised beaker. Variations from 5 grams need not exceed + or − 0.1 gram.

2. Rapidity in saponifying the fat and transferring the saponified fat to the distilling flask.

3. No foreign substances such as alcohol or glycerine are used, either of which may effect the results.

4. The contamination with CO_2 is reduced to a minimum.

[1]The solution of potassium hydrate is that of the A. O. A. C. method, page 247-23 (b), 1920, i.e. 100 grams of KOH to 58 cc. of distilled water and protect from CO_2.

"SYNTHETIC MILK" AS A BASIS FOR RESEARCH*

W. MANSFIELD CLARK

Hygienic Laboratory, United States Public Health Service, Washington, D. C.

Some time ago, while attempting to formulate the chief features of the acid-base equilibria of cows' milk, my attention was called to two distressing matters of very considerable importance to milk chemistry and milk technology. In the first place I found in the enormous literature no analysis of milk sufficiently complete and at the same time sufficiently well interpreted for my purpose. One of the several deficiencies lay in the interpretation of the origins of the phosphate appearing in the ash. Without definite and unequivocal knowledge of the distribution of phosphorus between the various organic forms and the phosphate entering the buffer action of milk, there could be no assurance in the treatment of buffer indices. The second matter was the lack of adequate information upon the conduct of even simple systems in which phosphate, calcium and citrate take part simultaneously in acid-base equilibria. Without this the next important step for milk chemistry—description of the conduct of such systems in the presence of milk proteins could not even be guessed.

The question of the analytical composition of milk has always been in some confusion because of the well known variability. This has needlessly overshadowed the advantages which could be gained from a complete physical and chemical analysis of one sample made with due consideration for the sources of error and with those types of duplication which would insure reasonable completeness for purposes of interpretation. The question of the conduct of simple systems involving citrate, phosphate and calcium may appear beside the point to one whose attention is focused upon the *variability* in the physical stability of cows' milk, for it may be argued that each milk is, in a sense, a new problem, and the study of variants is of more immediate impor-

* Published by permission of the Surgeon General, United States Public Health Service. Received for publication December 27, 1926.

tance to milk technology than is so academic a question as the conduct of a definite simple system remote from a complete milk. With such a point of view I would disagree most vigorously. The very fact of variability, both in chemical composition and in physical stability, precludes simultaneous studies having the completeness that the problem demands. One has only to inspect the existing data to realize that the correlations which it has been possible to make with the equipment and personnel of one laboratory are very limited, and that there lingers about every case the suspicion that undetected changes, as the investigator passes from case to case, may vitiate some of the sweeping conclusions that have been drawn.

Now there should be no fundamental difficulty in establishing a systematic survey of the citrate-calcium-phosphate equilibria mentioned. Much can be done with the *combination* of analytical measurements and hydrogen electrode measurements. Much more can be done if the calcium electrode can be developed. Still more will be done if the limitations in the classic equations for acid-base equilibria are recognized and dissociation constants and solubility products applicable to the environment of milk are determined and used in place of the constants determined for dilute and simple solutions.

In the meantime, it seems to me that some advances can be made with the use of a synthetic milk the gross composition of which can be determined by the ingredients used and the physical properties of which can be determined roughly at least by the manner of preparation.

Synthetic milks have been proposed before, more as curiosities than as a serious basis for experimentation. Porcher and Chevallier (1923) have cited these in their elaborate review of the composition of cows' milk. The fundamental difficulty with those proposed, as with Porcher and Chevallier's choice of terms in which to express the composition of true milk, is the use of specific salts which are assumed to exist as such in milk. In the first place, there is no justification whatever for neglecting the labile equilibria among the ions of the salts mentioned. The specification of definite salts gives an entirely erroneous impression. In

the second place, the fact of an irreversible shift in equilibria on the heat-treatment of milk shows definitely that a stable equilibrium in the original milk has not been reached and the attempt to duplicate the conditions of milk by the use of salt mixtures which will tend toward stabilized equilibrium before the final product is formed defeats one of the purposes to which a "synthetic" milk can be put.

Neglecting for the time being the minor constituents of true milk and the so-called excretory products found therein, we may arbitrarily assume the composition listed in table 1.

TABLE 1

Assumed composition of milk

	GRAMS PER LITER	EQUIVALENTS PER LITER	MOLS PER LITER
K_2O	1.80	0.0382	
Na_2O	0.72	0.0232	
CaO	1.78	0.0635	
MgO	0.30	0.0148	
Total		0.1397	
P_2O_5	1.50		0.0211(H_3PO_4)
Citric	2.00		0.0104
Cl	1.00		0.0282
SO_3	0.11		0.0014
Total CO_2			0.0050
Casein	28.0		
Albumin	7.2		
Other protein	0.2		

There are innumerable ways in which a system of the *composition* cited can be constructed. However, there is at least one very definite limitation upon practical ways and means. This is the excessively large proportion of calcium which must be handled with sulfate, citrate and phosphate. The very low solubility products for certain salts of calcium and these ions will result in premature precipitation and a final product not remotely resembling milk. Therefore, the problem is largely one of choosing that combination of salts and of choosing an order of mixing the solutions which may be presumed to furnish

the desired product. Whether or not this product represents the conditions of true milk remains to be seen. But it is the possibility of *controlled* variation in *composition* and in *physical state* that should make such a product a valuable material of research.

Of several possible schemes the following seems promising.

In its use the albumin and other proteins not casein were not available and were replaced by the equivalent of casein.

	Mols				
Solution 1	0.0074	MgO		0.298 grams	Dissolve with warming and dilute to 100 cc.
	0.0211	KH_2PO_4		0.873 grams	
	0.0104	Citric acid.	H_2O	2.185 grams	
Solution 2	0.0014	$CaCO_3$		0.140 grams	Dissolve cold. Dilute to 50 cc. and use while fresh
	0.0014	H_2SO_4	28 cc. N/10 H_2SO_4		
Solution 3	0.0141	$CaCl_2$	1.565 grams or preferably the equivalent of an analyzed solution. Dilute to 50 cc.		
Solution 4	0.0171	KOH	17.1 cc. N/1 KOH		Dilute to 50 cc.
	0.0232	NaOH	23.2 cc. N/1 NaOH		

Solution 5 (for 200 cc. "synthetic milk" in which casein represents total protein)

7.0 grams casein
10.0 grams lactose
} in 130 cc. N/20 $Ca(OH)_2$ solution

To prepare a lime-water as strong as N/20, thoroughly saturate the solution while it is cooled with surrounding ice. Add this slowly to the casein while the casein is being ground in a deep glass mortar. Finally stir with a motor-driven stirrer.

Add 10 cc. of solution 4. When the solution is free from suspended material add 10 grams lactose. As soon as this is dissolved add at once 10 cc. of solution 3 and 10 cc. of solution 2. Now set the motor at high speed and to the rapidly stirring solution add very slowly from the *fine* tip of a pipette 20 cc. of solution 1. When this addition is complete dilute the "milk" to 200 cc.

"Milks" prepared in this manner have the appearance of a very "blue" skim milk.

A sample in which the KOH-NaOH mixture was replaced by

NaOH and in which the casein employed was an unanalyzed sample of Harris casein "Nach Hammarsten" yielded the following data.

It was stable in the sense that no settling of the solid phase occurred when centrifuged one-half hour at 2500 r.p.m. in a large centrifuge (constants of centrifuge not determined). When sterilized by heat in a sealed tube a slight precipitation was observed after eight months. Analysis for N showed only 3 per cent casein indicating a high moisture content in the original

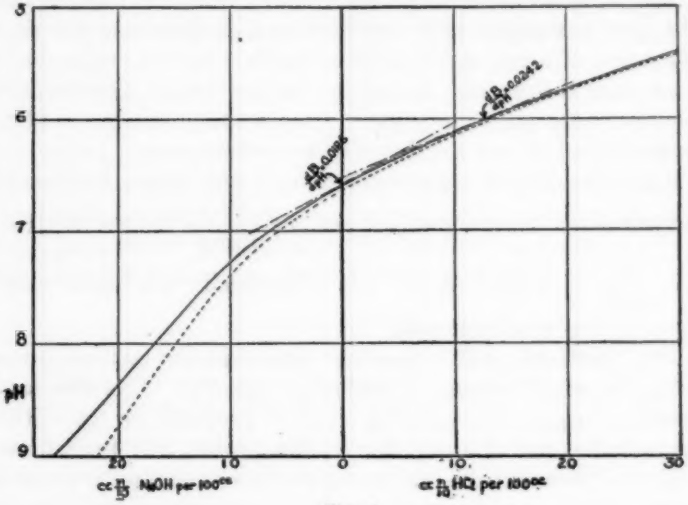

FIG. 1

casein and some little loss by adherence to the walls of the preparation vessel of a few grains of the coarse preparation. Total solids by customary procedure 8.44 per cent—representative value for solids not fat in real milk, is about 9. Ash by customary procedure 0.65—representative value for real milk 0.7. By reason of the absence of the minor constituents of milk the sample should show not only appreciably lower percentage composition but also an appreciably higher melting point. Two samples gave −0.521°C. as compared with −0.55°C. for true milk.

This synthetic milk formed rapidly with rennet a typical curd. Heating to boiling temperature in a test tube produced a more markedly milky appearance. The rennet action was then delayed.

No effort was made to equilibrate the solution with respect to normal CO_2 content. This can be done subsequent to the preparation. Measured as prepared and with a hydrogen electrode at 30°C. it gave an initial pH of 6.66. A representative value for milk is 6.6 ± 0.1.

The titration curve is shown in figure 1 by the dotted line. The good agreement between this titration curve and the titration curve of a real milk shown in figure 1 by full curve should not deceive the reader. In neither case are details of composition accurately known. Therefore it is only the gross aspects of the agreement which are worthy of present discussion.

Regarding the titration curve of true milk shown in figure 1 as typical, we find by graphic methods $\frac{dB}{dpH} = 0.0186$ at pH 6.6 and $\frac{dB}{dpH} = 0.0242$ at pH 6.0. Practically the same values apply to the "synthetic milk."

The "synthetic milk" described above should provide a material with which systematic studies can proceed. It is necessary, however, to give this warning. It will probably be found that the inclusion and the omission of the protein will furnish two radically different buffer values for the other components according to whether hydrogen electrode measurements are made on the system at equilibrium or before sufficient time for true equilibrium has elapsed. Compare, for instance, the curves for phosphate-Ca systems as described on the one hand by Davis, Oakes and Salisbury (1923) and, on the other hand, by Holt, LaMer and Chown (1925). The proteins must be regarded as not only contributing to the acid-base equilibria directly but as conditioning the state of solid phases. The latter effect will probably have to be duplicated by non-electrolytic colloids when the ionizable proteins are withdrawn.

The order in which components are mixed and the rate of

mixing will doubtless be found to influence the colloidal state of the product. In this connection it may be said that it seems highly improbable that certain of the dispersed but undoubtedly solid phases in which certain of the salts occur could be secreted as such by the mammary gland. Therefore, it is not too much to suggest that ingenious modifications of the rather crude scheme outlined above might lead to knowledge of components which it would be *possible* for the gland to secrete independently.

Of immediate importance is the use of such a controlled product in solving quantitatively the problem of the state of calcium and phosphate in milk. This is a matter of considerable importance not only to the technology of milk but to public health. We are informed that a very large proportion of our people show, in bone structure, evidences of incipient rickets and that the margin of safety in the assimilation of calcium and phosphate is extremely narrow. Improvement in the modification of cows' milk for infant feeding can hardly proceed with intelligence until the problem mentioned above is solved and solved in the exact and quantitative language of equilibrium data.

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THE RÔLE OF GELATIN IN ICE CREAM*

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The writers assume in this paper that a certain amount of viscosity is desirable. If it is desirable, what ingredient or group of ingredients is responsible for the viscosity as determined by the MacMichael Viscosimeter? Dahlberg of the New York Experiment Station, and others as well as this station, found that butterfat exerted some influence upon the viscosity of the mix. The milk-solids-not-fat exert more influence than does the butterfat but none of the dairy products exert the influence upon viscosity that does gelatin. As an illustration an ice cream mix containing no gelatin had an initial viscosity of 64.9 centipoises upon cooling to 20°C. Five-tenths per cent gelatin was added to part of the same mix and immediately cooled to 20°C. and the initial viscosity was 237 centipoises. An ice cream mix containing no gelatin will increase its viscosity. It is not unusual to obtain an increase of 500 per cent or greater in apparent viscosity due to the addition of gelatin.

Since gelatin seems to be the most important ingredient in obtaining viscosity, it will be the purpose of this paper to discuss the partial rôle of gelatin in an ice cream mix.

Gelatin is a protein and may be defined as the most typical of reversible colloids. Due to its chemical and physical properties, it is an excellent stabilizer of emulsions. The importance of stabilizing an ice cream mix is recognized, and it is being given a great deal of consideration.

There are two types of viscosity in an ice cream mix. One may be defined as real viscosity which is found in all solutions of crystalloids and colloids alike which have no connection with colloidal behavior. The other may be called apparent viscosity

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† Some of the data for this article were taken from the work of Mr. Milner presented as partial fulfillment of the requirements for his M.S. degree.

which is viscosity due to the swelling of submicroscopic solid particles in a solution. The apparent or colloidal viscosity has a greater order of magnitude than real viscosity.

The difference in the magnitude of casein and gelatin is due (1) to the fact that the gelatin possesses greater mechanism for increasing its relative volume in solution. The submicroscopic particles of gelatin or micellae occlude large amounts of water whereby the relative volume occupied by the gelatin is increased, these microscopic particles being the forerunner of a continuous gel to which the gelatin solution has a tendency to set.

There undoubtedly is a colloidal structure built up in an ice cream mix which has varying degrees of stability. This fact is borne out by Bogue (2) and others.

TABLE 1

Effect of quality of gelatin on viscosity

Number 30 certified wire

NUMBER	VISCOSITY (POISES)	
	Twenty-four hours	Forty-eight hours
1	2.60	9.36
2	9.36	15.08
3	13.52	26.00
4	28.60	Set*

* Too heavy to run.

The viscosity of a crystalloid solution is dependent only upon concentrations and temperature while in the case of colloidal viscosity we have quality, concentration, temperature, time and agitation. In the case of an ice cream mix the concentration is largely dependent upon the quality of the gelatin used.

Bogue states that the gel consistency is proportional to the undergrade protein present in the gelatin.

The quality of the gelatin has much to do with the viscosity (3) as shown by table 1.

The gelatins used above represented four grades and had a large range of gel strength from poor to excellent. The composition of the mixes in all cases was the same—11.00 fat and 36.84 total solids, 0.5 per cent gelatin in all four mixes.

Hatchesk states that the formation of a gel structure will vary with the brand and grade of gelatin. Less than 0.25 per cent will not set even at 0°C.

EFFECT OF AMOUNT OF GELATIN ON VISCOSITY

Our work on gelatin concentration bears out the work of Downey and others in proving that with each increase in amount of gelatin there was an increase in viscosity. The increase in viscosity was not nearly so great in smaller quantities (less than 0.4 per cent) as above. However, this depends largely upon the grade of gelatin used. (See table 2.)

TABLE 2

Effect of varying amounts of gelatin

34 wire

TIME AGED	AMOUNT OF GELATIN	VISCOSITY CENTIPOISE
hours	*per cent*	
24	0.2	64.6
24	0.3	102
24	0.4	428
24	0.5	1224
24	0.6	2892

This particular mix contained 10.78 per cent fat, 36.79 per cent total solids, to which gelatin was added in varying amounts (0.2 to 0.6 per cent).

The gelatin used in this mix was one of the better grades of gelatin.

The temperature of the mix during the aging period is important if the maximum amount of viscosity (all other factors being constant) is to remain in the finished ice cream. The colloidal structure derived from gelatin is very unstable under certain conditions. Von Schraeder (8) points out that the viscosity of a gelatin solution increases 0.1 per cent in sixty minutes, while by lowering the temperature to 24°C. there is an increase in sixty minutes of 1.5 per cent, and at 21°C. there is a 750 per cent increase in sixty minutes. As the temperature decreases the

threads lengthen and at the same time the water adsorbing power increases. This accounts for the rapid increase in viscosity with decreased temperature.

The increase in rigidity of the gelatin structure will depend upon the amount of the free solvent in the interfaces and the amount of solvent that has been taken up in the hydrated and imbibed condition.

The time required to age a mix properly is not the same for all mixes. If a poor grade of gelatin is used more time must be allowed for the gelatin to hydrate and the viscosity to develop than with a good grade of gelatin.

If viscosity is such an important factor and gelatin is the most important factor in obtaining viscosity, what, then, are some of the characteristics of gelatin that tend to stabilize viscosity?

Some authorities state that a good gelatin should make up a clear solution. Our study of gelatin does not indicate that color bears any great definite relation to jell strength, solubility, rate of setting or odor.

We find gelatins on the market varying in pH that make up clear solutions in water and by changing their pH they become turbid upon approaching the isoelectric point. We may take a turbid gelatin and make it clear by a change in pH away from the isoelectric point. By altering the pH away from the isoelectric point, we weaken the gel strength and viscosity. This is accounted for by Bogue (7) who states "That the greatest opacity results from large aggregates of less swollen particles. This maximum of opacity occurs at the isoelectric point. Any decrease in size of the aggregates or increase in hydration results in greater clarity or transparency of the solution."

This means that almost any gelatin may be made clear or turbid by altering its pH away from or toward its isoelectric point.

Table 3 illustrates the effect of the pH on the clarity of the gelatin solution.

We have found at least one exception which was undoubtedly due to the partial removal of the turbidity by the process of manufacture which does not necessarily improve the quality of

the gelatin for the manufacture of ice cream, though it does improve its general appearance in a water solution which is the way we have come to know gelatins.

The gel strength is influenced markedly by the pH of the gelatin as shown by the inversion test. Bogue (9) points out that the maximum gel consistency is at the pH of 4.0 to 4.5. In our work we used 1.5 per cent gelatin in water solution. The writers found that the gel consistency increased as the gelatin solution approached the isoelectric point. A weak gelatin may be strengthened if it has, for illustration, a pH of 7.0 or 3.0 by changing it to approximately 4.7. This does not mean that it would be possible to take a poor grade gelatin and by changing

TABLE 3

Effect of the pH on the clarity of the gelatin solution

SAMPLE NUMBER	pH	COLOR
1	7.0	Clear
1	4.7	Turbid
1	3.0	Clear
4	5.3	Turbid (slightly)
4	4.7	Turbid
4	3.0	Clear
2	6.6	Clear
2	4.7	Turbid
2	3.0	Clear

the pH make a high grade gel, although it may be improved somewhat. The odor of gelatin in water solution warmed to approximately 140° to 160°F. will indicate somewhat the grade.

Some gelatins were found to be apparently free of liquefying and putrefactive organisms but the pH was so high that they would not grow, and by changing the pH to approximately that of an ice cream mix these same gelatins showed the presence of large numbers. Some gelatins are sold to the trade with a pH so that those undesirable types would develop upon incubation in a water solution, while others would not.

Since the strongest gel is obtained when the gel is at a pH of approximately 4.7 and an ice cream mix under factory conditions

is approximately 6.30 (we realize that this figure varies slightly, depending upon source and quality of products used) the question arises whether the large reservoir of salts in the mix is sufficient to change the pH of the gelatin without materially changing the pH of the ice cream mix. If this is so, then gelatin should be purchased upon a little different basis than in the past.

TABLE 4

Effect of pH of gelatin upon pH of mix

MIX NUMBER	SAMPLE	pH OF GELATIN	pH OF MIX
A	1	7.6	6.27
A	2	3.8	6.23
A	3	3.8	6.17
A	4	4.0	6.18
A	5	No gelatin	6.24
B	6	4.7	6.42
B	7	3.0	6.21
B	8	6.3	6.25
B	9	8.0	6.30
B	10	No gelatin	6.31
C	11	4.2	6.33
C	12	7.0	6.37
C	13	3.8	6.23
C	14	5.8	6.26
C	15	No gelatin	6.30
D	16	3.8	6.27
D	17	7.0	6.33
D	18	4.0	6.26
D	19	7.6	6.28
D	20	No gelatin	6.29

As would be expected, the usual range in the pH value of gelatin affects but little the pH of an ice cream mix. The effect, if any, is of no commercial importance.

By referring to table 4, we conclude that the gelatin, regardless of its pH, changes to that of the ice cream mix. Undoubtedly there would be present in the average ice cream mix sufficient salts to change the pH of the gelatin to that of the mix. In table 4, each mix was divided into five parts and 0.5 per cent gelatin added with varying pH values to four of them, and to the fifth, no gelatin was added. All of the above readings repre-

sent four or more pH determinations. The variations in a mix with different gelatins, while excessive in some types of solutions, are not considered excessive by the authors, due to the complexity of an ice cream mix.

The pH determinations were made by use of the Type "K" Potentiometer using a Clark cell, the sample being bathed with a flow of hydrogen generated by hydrolysis. Uniform technique was followed in all samples. The pH of gelatins can easily and accurately be determined by the colorimetric method as described by Brown (4).

Many authorities are of the opinion that good gelatin aids in preventing the formation of ice crystals. It has been the writers' observation that the gold number is of little value in the evaluation of the quality of gelatin. It has been our experience that ice cream containing no gelatin has a greater tendency to form ice crystals. The concentration and quality of the gelatin has much to do with this phenomena. It was partially explained by Menz (5) when he showed that the protective action of gelatin is increased as the concentration is decreased. The work of Menz was corroborated by Elliott and Sheppard (6). Their conclusions were that the protective action is dependent upon the concentration of amicrous present in the solution.

SUMMARY

1. Gelatin seems to be the most important ingredient in obtaining viscosity as determined by the MacMichael.

2. Two types of viscosities are present in an ice cream mix which may be defined as real and apparent. The latter is very unstable under certain conditions.

3. The quality of gelatin is largely responsible for the amount of viscosity that may develop during aging.

4. The color of the gelatin and water solution does not necessarily indicate the quality of the gelatin.

5. The gel strength of the gelatin is influenced markedly by the pH.

6. Regardless of the initial pH of the gelatin, it assumes the pH of the ice cream mix probably due to the reservoir of salts and the small quantity of gelatin added (0.3 of 0.5 of 1 per cent).

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STUDIES ON YEASTS IN DAIRY PRODUCTS

III. THE PINK YEASTS COMMON IN MILK AND CREAM*

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INTRODUCTION

The yeasts forming pink colonies are very conspicuous among the organisms isolated from dairy products. While there is considerable variation in the color produced, this does not ordinarily extend to the point of questionable color production; occasionally a subsurface colony or even a young surface colony on certain media may fail to show a definite pink color, especially on heavily seeded plates, but usually color is produced in such amounts that it is readily recognized whether the colony develops on a plate or on some dairy product. Except for the pink yeasts, color production among the yeasts present in dairy products is unusual; in the examination of many hundreds of plates poured from dairy products for the isolation of yeasts, colonies producing pronounced colors other than pink have been encountered only a very few times.

A study was made of a considerable number of pink yeasts isolated from dairy products and the results are herein reported. From the data it appears that there is a common type but that certain cultures show variations from this; accordingly the group is divided into types and each characterized.

DISTRIBUTION OF THE PINK YEASTS IN DAIRY PRODUCTS

The pink yeasts have been repeatedly isolated at the Iowa Agricultural Experiment Station from a wide variety of dairy products. Sweet cream coming in from the college cream routes or delivered by farmers often contains a few hundred and occa-

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sionally a few thousand per cubic centimeter. Sour cream nearly always contains these organisms and sometimes they are present in considerable numbers. Like many of the other yeasts, the pink forms are capable of withstanding considerable amounts of acid and accordingly they sometimes appear on the surface of sour milk or cream or on soft cheese as distinct colonies that are usually round, smooth-edged and considerably raised. In some instances they occur singly or at least in small numbers while in others there may be a considerable number of colonies irregularly arranged or in a very distinct row or series of rows; the rows of colonies are undoubtedly due to a breaking up of a clump in quite the way a row of colonies occurs in an agar plate or, in the case of soft cheese, to contamination from a paddle used in handling the product.

Although pink yeasts have been repeatedly isolated from butter, colonies have never been observed on this product. The pink yeasts are undoubtedly present in butter because of their distribution about the creameries and their ability to withstand an unfavorable environment. While it is possible that they might be capable of forming colonies in butter under certain conditions these are apparently not usually provided.

GENERAL CHARACTERS OF THE PINK YEASTS

The pink yeasts isolated from dairy products have certain characteristics aside from the color produced in colonies on plates or other materials.

Changes are produced in milk rather slowly even in the presence of large amounts of growth as evidenced by the mass of pink color in a ring at the surface of the milk and in the sediment at the bottom. The production of a large amount of growth with comparatively little change in the milk in which this growth occurs is rather usual among the yeasts found in dairy products and is well illustrated by the pink type. After a considerable time milk in which the pink yeasts are growing coagulates and accompanying this there is more or less reduction of the litmus, particularly at the bottom of the tubes, while the color remaining indicates that

the reaction tends toward alkaline; certain cultures then digest the milk, and this digestion may eventually be very complete.

The temperature requirements of the pink yeasts are also of interest. These organisms in general do better at 21°C. than they do at 37°C., and some fail to grow at all at the latter temperature. Other groups of yeasts also seem to have their growth seriously interfered with at temperatures that are unusually favorable for many types of bacteria so that this relationship is not characteristic of the pink yeasts alone.

The colonies produced are usually approximately round on the surface of the plating medium and round or elliptical beneath the surface. Both the surface and subsurface colonies are quite smooth-edged and in the subsurface colonies there is no development of fingers or mycelium-like structures that project out into the medium and suggest mold colonies as is the case with many yeasts that give a typical yeast-like surface growth. Sometimes in old plates some of the colonies develop fissures from which cells grow out and form a structure suggesting a new colony, but this secondary growth is again smooth-edged; the breaks in the edge of the old colonies are more likely to occur in subsurface colonies than in surface colonies and are most frequent along the sides of elliptical colonies.

CULTURES STUDIED

Approximately 90 cultures of pink yeasts were studied in more or less detail. These were largely secured from milk, cream, soft cheese and butter obtained in the vicinity of Ames; many of the cultures were picked from plates poured for the purpose of total yeast and mold counts. For the most part only one culture was selected from a plate so that the cultures studied came from a considerable number of samples of various materials. A transfer of a culture isolated from oysters by Hunter[1] was supplied by Dr. Thom and included in the cultures investigated.

[1] A pink yeast causing spoilage in oysters. U. S. Dept. Agr. Bul. 819, March, 1920.

VARIATIONS IN THE CULTURES STUDIED

A very large per cent of the pink yeasts studied very evidently belong to the same species which is referred to as the common type. Variations in the intensity of the color produced, in the rate of growth at different temperatures, etc. were noted among these cultures, as would be anticipated, but undoubtedly these simply represent variations within the species and are of little significance from the standpoint of classification except to help establish the variations to be expected. However more pronounced variations which follow through several characters were also noted with a few cultures. Two groups of these pronounced variations were encountered and the organisms showing them were considered to constitute two types distinct from the common type; these variations are given in detail following the description of the common type of pink yeast.

DESIGNATION OF THE COMMON PINK YEAST

The common type of pink yeast studied apparently resembles very closely the organism referred to as Torula glutinis or Cryptococcus glutinis. However, Guilliermond[2] has pointed out that, "Torula glutinis does not possess a very characteristic appearance and a series of related yeasts have been described under this name"; a consideration of the literature shows that this statement is amply justified. Golden and Ferris[3] in discussing T. glutinis state that, "An error seems to have crept into the description of this species in regard to its size, the other characters being practically the same in all the descriptions."

It should perhaps be recognized that in the determination of certain characters there are likely to be discrepancies in the results reported by different laboratories due to variations in the methods even when the same cultures are used and it is possible that these variations would be even greater if different cultures of what should be considered as the same species were employed. The pink yeasts, although giving a rapid growth, bring about few

[2] The Yeasts. Translation by Tanner, p. 318.
[3] Red Yeasts. Bot. Gaz., 1898, xxv, p. 45.

bio-chemical changes and these only very slowly, so that the period over which the observations are continued may be an important point. Hunter reported, "No coagulation nor peptonization" of litmus milk for the yeast he isolated from oysters, while the culture from Hunter's isolations that was included with those discussed herein gave, after a rather long period, both coagulation and digestion.

It seems advisable to consider the common yeast that was found in dairy products examined as Torula glutinis and to depend on the use of this name and additional investigations of the pink yeasts to develop a definite meaning and significance for it.

DESCRIPTION OF THE COMMON PINK YEAST—TORULA GLUTINIS

Morphology

Form. The cells were oval. Although spherical appearing cells were frequently noted these were presumed to be oval cells seen from an end. Elongated, thread-like cells were not observed, even in subsurface colonies.

Size. The cells varied greatly in size with the length usually ranging from 2.5 to 4.0 microns and the width from 1.5 to 2.5 microns.

Arrangement. Most of the cells were single, although budding was common. Buds were most numerous in young cultures but were also observed in cultures several weeks old. Only one bud was found attached to each mother cell and this always appeared at the smaller end. It was unusual to find cells attached unless one was somewhat smaller than the other.

Staining reaction. The cells stained readily. Most of the cells in young cultures from both liquid and solid media were gram positive while a few were gram negative. Older cultures showed a greater proportion of the cells gram negative.

Spore formation. Spore formation was never observed.

Cultural characteristics

Whey agar slope. At room temperature growth was quite rapid; it was usually distinct in twenty-four hours and increased rapidly for several days. The growth was pink, filiform, raised, glistening, and non-

viscous. Certain variations occurred in the rate of growth and in the intensity of the color produced.

Beef infusion agar slope. Growth was quite like that on whey agar in both character and amount.

Beef extract agar slope. Growth was quite like that on whey agar in both character and amount.

Whey agar stab. At room temperature growth, after twenty-four hours, was distinct, especially at the surface, and increased rapidly. The surface growth was pink, raised, smooth-edged, glistening and non-viscous. Along the stab the growth decreased rapidly from the surface and was filiform in character, with the color less pronounced than at the surface.

Whey agar plate colony. At room temperature colonies were frequently evident after twenty-four hours while after forty-eight hours the surface colonies were often up to 1 mm. in diameter and the sur-surface colonies about one-third of this. Growth continued rather rapidly. The surface colonies eventually became several millimeters in diameter and were round, pink, considerably raised, smooth-edged, glistening and non-viscous, while the subsurface colonies were round to ellipsoid, somewhat pink, and smooth-edged. No filaments or thread-like structures were observed.

Whey gelatin stab. Growth was essentially like that in a whey agar stab, with no evidence of liquefaction even after several weeks.

Bouillons. Plain bouillon and bouillons to which additions had been made showed a turbidity and frequently some pink sediment after twenty-four hours at room temperature. Growth increased and a heavy pink ring was often formed at the surface. Eventually the turbidity cleared. Certain sugars, particularly the monoses, seemed to favor growth to a certain extent.

Potato. At room temperature growth was evident after twenty-four hours and increased rapidly; it was raised, smooth-edged and decidedly pink. In very old cultures in which the potato had dried up the color seemed to be brown rather than pink.

Dunham's solution. Growth was evident as a fine turbidity after twenty-four hours at room temperature. As the growth continued a fine sediment developed and as this increased it became quite pink. The turbidity eventually cleared. Frequently there was a tendency to an increased growth at the surface but no distinct ring or pellicle formed.

Uschinsky's solution. Growth was comparatively slow but was evident as a distinct sediment after three days at room temperature. The

sediment increased in amount as the cultures aged and became pink in color.

Litmus milk. At room temperature growth was evident in two or three days as a pink sediment and a beginning ring at the surface, the ring often appearing rather granular. A slight reduction of the litmus at the bottom of the tube was sometimes observed. As the cultures aged the ring and sediment increased in amount. Eventually the milk coagulated but this usually required several weeks; the coagulation commonly began at the bottom. Most of the coagulated cultures showed considerable reduction in the lower portion of the tubes with a pronounced blue color at the surface.

Soured milk. Growth was rapid on the surface of soured milk at room temperature. The growth either in stabs or on slopes resembled the growth on whey agar in character and amount.

Bio-chemical features

Gas production. Gas was never observed either in milk or in bouillons to which various additions had been made.

Oxygen relation. The general character of the growth in various cultures indicated the preference of the organism for an abundant air supply.

Reaction change. The organisms apparently produced changes in reaction only slowly. Milk became alkaline after considerable periods. Neutral bouillons to which mannitol, salicin or inulin had been added, were alkaline after seven days at room temperature. Bouillons with glucose or levulose gave acidities from 0.4 to 0.9 per cent N/1 acid while bouillons with galactose, maltose, sucrose or lactose showed still lower acidities.

ADDITIONAL TYPES OF PINK YEASTS

The types of pink yeasts which show pronounced variations that follow through several characters are as follows:

Type A. Torula rubicunda. Several cultures of this type were secured from a number of different sources. The first culture was obtained from a sample of well water. The culture isolated by Hunter was also grouped here. Later a number of cultures isolated in a dairy barn were included. The variations distinguishing this type from the common type are:

1. The pink color produced was very intense. This was true with all the different media used and involved both the surface growth on solid media and the sediment in liquid media.

2. The cells were larger than those of the cultures of the common type, and this greater size occurred with various media. The width of the cells usually varied from 2.0 to 3.3 microns and the length from 2.5 to 6.0 microns.

3. In very old milk cultures there was commonly a digestion of the casein. This digestion never appeared until the cultures were several weeks old and sometimes it failed to appear at all, due possibly to the drying up of the milk. Digestion was never noted in the cultures of T. glutinis.

Type B. Torula paraglutinis. Only two cultures of this type were found and these came from the same sample of cream. The variations distinguishing this type from the common type are:

1. With all the different media used growth was quite slow. On whey agar at room temperature about three days were required for the colonies to be evident while with the usual cultures colonies were evident in one day and in three days were often up to 2 mm. in diameter.

2. In liquid media the growth was more flocculent than turbid, while turbidity was common with T. glutinis in liquid media.

3. With all media the color tended to be slight, and with certain sediments developed in liquid media the pink color was so slight as to be scarcely detectable.

4. On agars the growth tended to be viscous.

5. At 30°C. growth was negative or questionable. With the usual cultures growth was very rapid at this temperature.

DESIGNATION OF THE PINK YEASTS SHOWING VARIATIONS FROM THE COMMON TYPE

Although Type A and Type B show many of the general characters of the common type of pink yeasts they seem to constitute distinct species for which descriptions could not be found in the literature. With both of these types the number of cultures studied is too small to make possible a description that shows the variations to be expected but because of their relationship to the common type of pink yeast it seemed advisable to

designate them tentatively. The name Torula rubicunda is accordingly proposed for Type A and Torula paraglutinis for Type B.

SUMMARY

Approximately 90 cultures of pink yeasts were isolated and studied in more or less detail. These came largely from milk, cream, soft cheese and butter obtained in the vicinity of Ames. It was found that most of the cultures belonged to a common type, which is considered to be Torula glutinis, although it is smaller than the dimensions given in most of the descriptions of this organism. Two other types were encountered in small numbers and named Torula rubicunda and Torula paraglutinis to make possible their distinction from the common type. A description of Torula glutinis is given together with a list of the variations from this type shown by Torula rubicunda and by Torula paraglutinis.

SEPARATION OF CANE SUGAR FROM WATER ICE*

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Makers of ice-cream products occasionally find a water ice in which the cane sugar has crystallized, making the product hard, chalky in appearance, and unpalatable. The researches described in this paper may help those who have experienced such a difficulty, since the conditions governing the crystallization of sucrose from frozen solutions have been established with considerable exactness.

In considering this problem, the solubility, temperature, and freezing-point relationships of cane-sugar solutions of varying concentrations should be established if possible. Those temperatures incident to normal ice cream work are, of course, of primary interest.

Upon the cooling of an unsaturated dilute solution to the proper point, ice separates from it. This separation concentrates the sugar in the unfrozen part, and as the temperature lowers this concentration increases. By plotting temperatures and concentrations incident to these temperatures, the freezing-point curve is obtained. Similarly, if a highly concentrated solution is cooled, its saturation point is reached, after which, under suitable conditions, sugar crystals are thrown out of solution. This process lessens the concentration of the sugar in the water, and increasingly lower temperatures are necessary to force out further crystals. These data, plotted in a manner similar to the freezing point data, yield the solubility curve. These curves approach each other, and at their point of coincidence is found a solution saturated to sugar and in equilibrium with ice. Under ideal conditions further abstraction of heat from this mass does not lower the temperature but brings about the separation of both sugar and ice until the mass has become solid, after which the temperature is of course lowered with further removal of heat.

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This point of complete solidification is known as the eutectic point. It is at once evident that if a sugar solution is frozen of a concentration less than that represented by the eutectic, sugar can not be separated from it until this eutectic temperature is reached, because the solution will not until then become saturated with sugar. This means, then, that if water ice is not brought to a temperature lower than the eutectic for cane sugar, the sugar can not separate. In other words, if the ice is not stored at too low a temperature this difficulty can not be experienced. The purpose of the work outlined in this paper is to determine the eutectic temperatures for cane sugar.

Guthrie (1) in a paper published about 30 years ago states that this eutectic temperature is about −8.5°C. (16.7°F.). He also gives some data on the freezing points of different sugar solutions. Pickering (2) has also given some freezing-point data. Certain difficulties are encountered in determining the freezing points of the highly viscous cane-sugar solutions; and since the measurements recorded in these papers were made before the development of accurate methods, Mr. P. N. Peter of these laboratories has redetermined the position of this curve. His data are plotted. There is a very fair agreement among the data of the three men.

A similar survey of the work published on cane-sugar solubility failed to disclose any measurements carried out at the lower temperatures. Such measurements also offer considerable difficulty owing to the high viscosity of these concentrated solutions, with the resultant tendency to supercooling, and especially with the remarkable tendency of cane sugar to resist crystallization.

Work is in progress to determine definitely the solubility of cane sugar in water at the lower temperatures. For the purpose of this paper, however, it will be sufficient to determine empirically the solubility of cane sugar, since there are other means of locating the eutectic of the cane-sugar solution.

Where S is the solubility of sugar in 100 parts of water and t is the temperature in degrees Centigrade, the empirical formula (3) is:

$$S = \frac{28162}{157.97 - t}$$

The solubility curve of cane sugar calculated from this formula is given in figure 1, together with the freezing-point curves. It will be seen that the solubility and freezing-point curves intersect at a temperature of about −12°C. (10.4°F.). This temperature, lower than that given by Guthrie, is sufficiently low for the holding of water ices, at least those of moderate sugar content.

It now remains to determine the accuracy of the figure. A rather simple method, and one often used in metallurgical work, is to determine the rate of cooling of a sugar solution with the idea that if sugar separates from it because of heat liberation incident to this reaction, the rate of cooling will also change. The reverse process can also be carried out, namely, to warm the

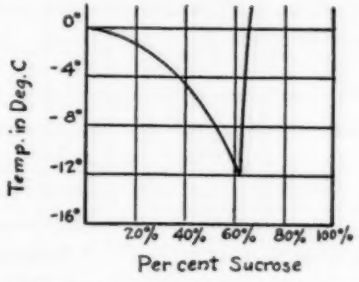

FIG. 1. SOLUBILITY AND FREEZING POINT CURVES OF SUCROSE-WATER SOLUTIONS

frozen mass and determine the rate of warming. This procedure is perhaps the better one, since it is possible to supercool such a solution, and the sugar separation may not come at the true eutectic. This is not the case with the warming process since, if equilibrium is always attained in the separation of the ice (and there is every reason to believe that it is), the sugar can not dissolve until the eutectic is reached. In this work, as clear-cut results are not to be expected as are usually obtained in metallurgical studies because of the comparatively poor heat conductivity of the sugar solutions and the slow crystallization or resolution of the cane sugar. The method is of value, however, as the results show.

The experimental procedure was as follows: About 20 pounds

of cane-sugar solution containing 25 per cent sugar was placed in the hardening room after freezing in the regular power ice-cream freezer. About 0.5 per cent gelatin had been added, which was sufficient to permit whipping in the freezer. In a liquid mass the ice crystals could concentrate on the surface of the mix, while here the gelatin gave the mass enough structure so that no such separation could take place. The temperatures in the center of the can were measured with a thermocouple with an accuracy of about one-fiftieth of a degree, the temperature being measured at varying intervals from five minutes to one-half hour, depending upon the rapidity with which the material was found to be cool-

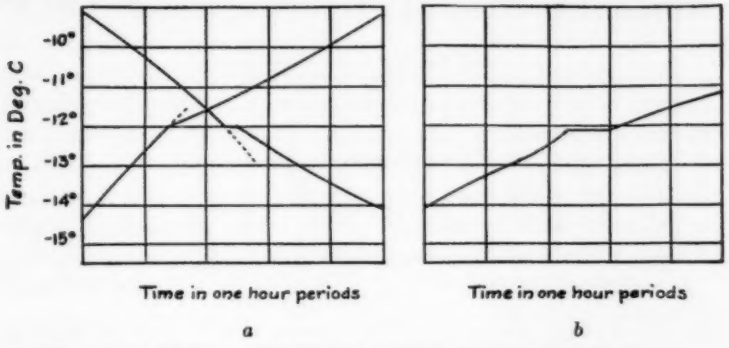

FIG. 2. *a*, COOLING AND WARMING CURVES OF WATER ICE; *b*, WARMING CURVES OF ICE CREAM

ing. The curve is given in figure 2*a*. It will be noticed that there is a distinct change of slope at a temperature of about −12°C. (10.4°F.). The warming curve also shows a change of slope at this temperature, and it is justifiable to conclude that this is the true eutectic temperature for cane-sugar solutions in water. In this particular instance, while some of the sugar had crystallized, the sugar separation was only partial, since the author was able to remove a small portion of unfrozen material from the mass when it was at a temperature of about 0°F., which substance showed a concentration of 73 per cent sugar. This represents a point in the freezing-point curve in the supersaturated

area, which is very good proof that ice crystallization reached equilibrium at all times during the experiment.

Figure 2*b* also shows the warming curve for an ice-cream mix. This curve is given because it shows that with a considerable sugar separation the temperature of the mass remained unchanged for nearly three-quarters of an hour. The discussion of the separation of milk sugar and cane sugar from ice cream will, however, be the subject of another paper. It should be noted here that the crystallization, due to the presence of substances other than water and sugar, takes place at a slightly lower temperature in the ice-cream mix than in the water ice.

In conclusion it may be stated that if water ice is not cooled to a temperature equal or below −12°C. (10.4°F.), sucrose can not separate from the solution. It may be well to emphasize that the value of this temperature is in no way dependent upon the original concentration of the sugar solution, also that it will in all probability be altered but little by the flavoring material added to the water ice. The probability of crystallization after this temperature is attained will undoubtedly be greater with the more highly concentrated sugar solutions.

The author is indebted to Mr. O. E. Williams for aid and advice with the work.

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BUFFERS OF MILK AND BUFFER VALUE*

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Since buffers are considered as substances which, by their presence in solution, increase the amount of alkali or acid that must be added to cause unit change in pH, the question of their occurrence in milk would be important. Clark (1) gives the following with regard to buffers: "By buffer action, we mean the ability of a solution to resist change in pH through the addition or loss of alkali or acid."

Among the agents, which act well as buffers, are mixtures of weak acids or bases and their salts. Milk might be expected to have a definite buffer value, due probably to the combined effects of salts and protein matter present. Van Slyke (2) has shown that human blood has a rather definite buffer value of 0.0228 at pH of 7.4. Milk is known to bear a close relationship to blood. Both have a small amount of sugars and protein in true or colloidal solution. These together with the inorganic salts are definite enough in quantity so that both milk and blood exhibit a definite osmotic pressure as measured by the freezing point. Since blood is well buffered against rapid change in pH it might be considered that milk would also show a definite buffering effect.

The importance of the buffer value of milk may be illustrated in a number of ways. In bacteriological experiments using milk as a media, if milk should have a constant or uniform buffer value, the rate of growth of bacteria could be observed by noting the acidity produced. However, if milk does not possess a very uniform buffer value at any pH range (5), the extent of growth could not be accurately made without, in each case, determining the buffer value of milk used. In a similar way the quantitative

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estimation of the various sugars in milk (3, 4), in which acidity produced by microörganisms is taken as the measure of the amount of carbohydrate present, would be very difficult, unless it were known that milk had a definite buffer value. Also, if different milk samples possessed widely varying buffer values, it would be possible for a highly buffered milk to remain sweet longer than a more poorly buffered one. It would necessarily take a larger amount of lactic acid to produce the same change in pH in the former as compared to the latter milk. Again, indefinite as the chemistry of milk may be, a number of investigators (6, 7, 8, 9, 10) seem to agree that acid phosphates and casein are responsible for the greatest part of the original acidity of milk. Granting these to be the chief causes of the original acidity of milk, it is reasonable to assume that a high acid milk should exhibit a greater buffer action than a low acid milk (11) because of the greater phosphate and casein content.

In this work Van Slyke's (2) method of measuring buffer values was used. A convenient quantity of the solution to be tested was taken and the pH measured. Strong acid or alkali, usually 0.5 N KOH or HCl, was added in small measured amounts and the pH measured after each addition. The buffer value was obtained from these data by plotting the amount of acid or alkali used against the change in pH produced. If too large a range is not taken, this average buffer value represents closely the actual buffer value at any point in that small range. As a numerical measure of the buffer value β of a solution, Van Slyke (2) proposes to take the number of gram equivalents of strong alkali or acid taken up by a liter of solution per unit change in pH. Since β varies with varying pH, the value at any given pH is defined by the ratio $dB/d\text{pH} = \beta$ expressing the relationship between the increment (in gram equivalents per liter) of strong base or acid B added to a buffer solution and the resultant increment in pH.

An example of the calculation of β is given to illustrate the method of evaluating the constant.

From figure 1 the following values may be taken from the

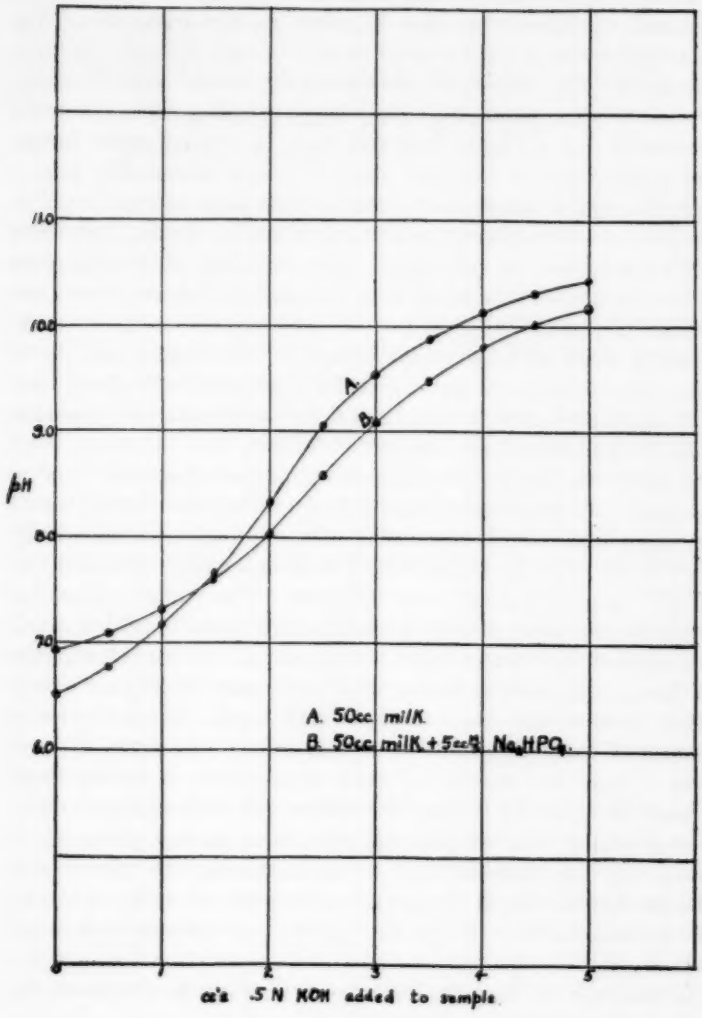
11.0
10.0
9.0
pH
8.0
7.0
6.0
A
B
A. 50cc. milK
B. 50cc. milK + 5cc. Na_2HPO_4.
0
1
2
3
4
5
cc's .5 N KOH added to sample

FIG. 1

curve A. For the pH range 7.0 to 7.5 it required 0.58 cc. of 0.5 N KOH to cause a change of 0.5 pH in a 50 cc. sample of milk.

$$\frac{dB}{d\text{pH}} = \frac{\left(\frac{0.58}{1000} \times 0.5\right)}{0.5}\left(\frac{1000}{50}\right) = 0.0116 = \beta$$

where dB is the fraction of a gram equivalent of KOH added, dpH = 0.5 and 1000/50 is the ratio to change the experimental results from a 50 cc. sample of buffer solution to a 1 liter quantity. This value β is always a positive one, for if we add strong acid, it is equivalent to adding a negative amount of base. The pH increase is also negative, hence β is positive. In these terms a solution has a buffer value of 1 if a liter of that solution requires 1 gram equivalent of strong acid or base per unit change of pH.

The apparatus employed was the regular Leeds and Northrup potentiometer using the customary set-up for H-ion determination. The Hildebrand hydrogen electrode and a normal calomel electrode were used in all of the determinations. Additions of acid or base were made from a calibrated burette having a long, fine, capillary tip (12) reaching beneath the surface of the liquid at a point near the stirrer. This, together with the uniform rate of stirring provided by the motor-driven stirrer, prevented the possibilities of any "local action," and insured a rapid and even distribution throughout the liquid. Best results were obtained with a moderate rate of stirring with a stirrer shaped so that there was little churning action.

RELATIONSHIP BETWEEN pH REGION AND BUFFER VALUE

Table 1 gives a summary of the buffer values calculated from the data obtained over a pH range varying from pH 4.5 to pH 10.0. It may be observed from the table that the buffer value is not very definite in the acid range. In the alkaline range the value is more constant giving in the range pH 8.5 to 9.0 a value of 0.0067. The values given in table 1 as well as all values considered were on unpasteurized milk.

EFFECT OF ADDED WATER ON BUFFER VALUE

If we assume a definite buffer value in the pH range 8.5 to 9.0, the determination of this value would serve as a method of detecting added water in milk providing a variation from this value would be produced by dilution with water. Table 2

TABLE 1

Variation of buffer value with pH region

pH ZONE	AVERAGE	LIMITING VALUES	VARIATION FROM AVERAGE	
4.5 to 5.0	0.03250	0.0379 to 0.0264	+0.0054	−0.0061
5.0 to 5.5	0.03255	0.0392 to 0.0280	0.0067	0.0045
5.5 to 6.0	0.02033	0.0234 to 0.0139	0.0031	0.0064
6.0 to 6.5	0.01720	0.0200 to 0.0148	0.0028	0.0024
6.5 to 7.0	0.01574	0.0165 to 0.0143	0.0008	0.0014
7.0 to 7.5	0.01200	0.0130 to 0.0112	0.0010	0.0008
7.5 to 8.0	0.00796	0.0094 to 0.0069	0.0013	0.0010
8.0 to 8.5	0.00635	0.0082 to 0.0060	0.0018	0.0004
8.5 to 9.0	0.00670	0.0074 to 0.0064	0.0007	0.0003
9.0 to 9.5	0.00950	0.0118 to 0.0082	0.0023	0.0013
9.5 to 10.0	0.01520	0.0174 to 0.0134	0.0022	0.0018

TABLE 2

Effect of added water on buffer value

	SAMPLE 1	SAMPLE 2	SAMPLE 3	SAMPLE 4	SAMPLE 5
Volume of water added to 50 cc. of milk.....	10 cc.	10 cc.	15 cc.	15 cc.	15 cc.
For pH range 6.5 to 7.0.....	0.0160	0.0060	0.0165	0.0165	0.0174
7.0 to 7.5.....	0.0114	0.0115	0.0123	0.0116	0.0119
7.5 to 8.0.....	0.0080	0.0079	0.0075	0.0080	0.0080
8.0 to 8.5.....	0.0060	0.0062	0.0072	0.0066	0.0064
8.5 to 8.9.....	0.0068	0.0068	0.0074	0.0070	0.0068
9.0 to 9.5.....	0.0096	0.0098	0.0100	0.0098	0.0102
9.5 to 10.0.....	0.0154	0.0158	0.0176	0.0170	0.0170

gives data from which the conclusion would be drawn that dilution of milk up to 30 per cent with water does not affect the buffer value. No error would then be introduced due to the dilution of the milk with the reagent required to bring the pH to a definite range.

BUFFER EFFECT DUE TO CASEIN

In the determining of the buffer values in the acid range, it was found that the casein was precipitated in the range pH 4.5 to 5.0, usually at about pH 4.7. No marked change in buffer value was observed in this range. Hence it would be concluded that the casein exerted very little influence as a buffer in this pH range in milk. Bailey and Peterson (13) noticed but little change in buffer values of flour extracts on the precipitation of the protein matter.

TABLE 3

Effect of added Na_2HPO_4 on buffer value

	SAMPLE 6	SAMPLE 7	SAMPLE 8	SAMPLE 9	SAMPLE 10	SAMPLE 11	SAMPLE 12	SAMPLE 13
Quantity of Na_2HPO_4 added to 50 cc. milk	5 cc. M/3 solution	5 cc. M/3 solution	5 cc. M/3 solution	2.5 cc. M/3 solution	2.5 cc. M/3 solution	2.5 cc. M/3 solution	1 cc. M/3 solution	1 cc. M/3 solution
pH range 6.5 to 7.0				0.0262	0.0240	0.0237	0.0192	0.0189
7.0 to 7.5	0.0222	0.0190	0.0212	0.0168	0.0170	0.0174	0.0138	0.0144
7.5 to 8.0	0.0122	0.0142	0.0132	0.0096	0.0108	0.0108	0.0092	0.0086
8.0 to 8.5	0.0092	0.0102	0.0090	0.0082	0.0084	0.0084	0.0074	0.0086
8.5 to 9.0	0.0094	0.0094	0.0094	0.0086	0.0088	0.0086	0.0078	0.0074
9.0 to 9.5	0.0130	0.0126	0.0122	0.0118	0.0118	0.0114	0.0114	
9.5 to 10.0	0.0190	0.0150	0.0200	0.0200	0.0196	0.0200	0.0172	0.0178
pH after addition of the Na_2HPO_4	6.924	6.917	6.916	6.681	6.741	6.741	6.668	6.650

BUFFER EFFECT DUE TO PHOSPHATES

In figure 1 are given the titration curves of 50 cc. of milk titrated with 0.5 N KOH and of 50 cc. of milk + 5 cc. M/3 Na_2HPO_4. The similarity of these curves to the curve obtained on titration of a solution of disodium phosphate is easily recognized, and suggests the possibility of the phosphates exerting a buffer effect in milk. To give information on this point, small quantities of Na_2HPO_4 were added to milk and the buffer values calculated over a range of pH 6.5 to 10.0. These values are shown in table 3. The average initial acidity of all samples of milk tested was between pH 6.500 and 6.630. By the addition of the phosphate the initial acidity of the milk was decreased.

The buffer value is also slightly increased depending upon the quantity of the phosphate added. For example, the average buffer value for the range 7.0 to 7.5 is 0.0120. The addition of 5 cc. M/3 Na_2HPO_4 increases β to 0.0212 while 2.5 cc. increases the value to 0.0170 and 1 cc. to 0.0140. Since the type of curve, as shown in figure 1, is the same and is only modified by having its values farther up on the scale, it indicates for this range a very close relation between phosphate and the buffer substances present in milk.

As Na_2HPO_4 in water solution hydrolyzes to give a basic reaction, that largely accounts for the increase in initial pH and very probably for the increased buffer action. It was thought possible that the results observed with Na_2HPO_4 might simply be a salt effect on the buffers contained in milk, that is, that any salt might modify the buffer values in the different ranges in somewhat the same manner. On the addition of KCl, $CaCl_2$, and $FeCl_3$, to samples of milk it was found that while the buffer value was affected, the results were neither regular nor in the same order as with the Na_2HPO_4. Hence the effect of Na_2HPO_4 would not be considered as a salt effect upon the buffers of milk.

CONCLUSIONS

1. The calculated buffer value of milk varies with the pH range and is the more definite at the range pH 8.5 to 9.0, with an average value of 0.0067.

2. Casein has very little effect as a buffer in the region of its iso-electric point, since the precipitation of the casein has no great influence upon the buffer value.

3. The phosphates of milk are at least among the most important buffers of milk. This is indicated by the fact that there is a great similarity between the titration curves of milk and Na_2HPO_4.

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A VOLUME WEIGHT STUDY OF ICE CREAM*

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INTRODUCTION

The problem concerning the shrinkage of ice cream upon its being dished from bulk can containers is an ever-present one whenever and wherever ice cream is sold in bulk to the consuming public. This problem is assuming greater importance daily as the ice cream industry is growing so rapidly. With its direct bearing upon the increasing agitation now in progress for a sales by weight program and for goods packaged at the manufacturer's plant, it has indeed reached a point of very vital significance to the industry.

It has long been observed that there is considerable shrinkage in volume when ice cream is dipped from a can. Numerous articles have been written and published upon this problem. The latest and most important publications are here given as references (1–8).

The purpose of this research was to ascertain the effect of various factors on shrinkage with a view of discovering possible preventive methods of diminishing shrinkage.

EXPERIMENTAL

Method

In the scientific study of any problem there must be a standard upon which to base results. This is particularly true of any work upon ice cream which is of such a complex nature. So

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† Acknowledgment is here made to Professor H. F. Judkins, former head of the Dairy Department, who originally proposed this problem and under whose guidance the major portion of this work was performed and to Professor J. H. Frandsen, present head of the department, for valuable suggestions and criticisms.

standard methods of procedure were employed throughout the entire work.

A standard mix containing 12 per cent fat, 10.5 per cent serum solids, 38 per cent total solids, 14.5 per cent sugar, 0.5 per cent gelatine and 0.5 per cent powdered egg yolk was used in all work except as otherwise noted in the text. The mix was made from fresh cream, skim milk and skim milk powder.

The average weight in pounds per gallon of the standard mixes was 9.16.

All freezing was done in a Fort Atkinson freezer of 10 gallons capacity. Fifty pounds of mix were used for each batch. The ice cream was drawn into 3-gallon cans. These cans were tared so that a definite check on the overrun could be obtained, for each can was reweighed after filling and the overrun thus accurately determined. The overruns thus determined were the ones used in all calculations.

An interesting result of this weighing of each can is observed when the overrun of the first, second, and third cans drawn are compared. The results show that in 65 per cent of the cases the second can was of higher overrun than the first can and in 30 per cent of the cases the second can was of lower overrun. With the third can the ratio was 50 per cent higher and 45 per cent lower overrun than in the first can. The can of the highest overrun was the first can in 9 per cent of the cases, the second can in 24 per cent of the cases and the third can in 18 per cent of the cases. The figures for the lowest overrun are 23 per cent for the first can, 7 per cent for the second and 19 per cent for the third. These figures are from observations on 58 batches which gave 174 cans.

The overrun was tested frequently during the whipping by running out the ice cream into a one pint cup measuring $2\frac{1}{16}$ inches deep by $4\frac{1}{16}$ inches in diameter. The excess ice cream was leveled off by means of a saptula. The large diameter of the cup permitted the ice cream ribbon to fold into the container with a minimum amount of included air holes. The cup and contents were weighed on beam scales and the overrun computed directly. As previously mentioned, the overrun was checked by weighing each individual can.

Before dishing, each can was removed from the hardening room and placed in a mechanically refrigerated cabinet where it was allowed to temper for at least twelve hours. Unless otherwise stated all cans were tempered and dished at 10°F. When removed from the cabinet, the can was immediately placed into an insulated jacket and remained therein during the entire period of dishing.

The temperature of the ice cream was taken with a low reading thermometer from time to time and no appreciable change in temperature was noted except in a few cases when the work was interrupted.

In all the work every factor was kept as nearly uniform and standard as was possible, varying but one factor at a time in order that its effect alone might be studied. The matter of personal equation was kept as nearly constant as could be desired by using great care in all phases of the work. The pressure exerted upon the dishing tool, the degree of heaping the disher, the pressure applied to the ice cream as it was introduced into the carton, all were carried out with extreme uniformity. Yet at the same time conditions were strived for which were as nearly as possible identical with those which would be met with in actual commercial work. The data for each can were never reviewed until after the entire can had been dished.

A. Size and type of dishing tool

In order to study the effect of the size and type of disher employed for the dishing of the ice cream, several sizes and types were used in these experiments. The regular self ejecting semispherical disher was used in most work. Wherever the word "disher" appears, this type of disher is indicated. The size number indicates the fraction of a quart contained in one level disherful.

When dishing the ice cream, the number of disherfuls per 3-gallon can was noted. Each disherful was placed upon a clean tared paper plate and weighed.

In all dishing, attempt was made not to get too many extra full dishers or too many with air spaces in them. To make a

solid, even, good looking ball such as commercial dealers would dish, the ice cream was pressed into the disher by a slight, yet consistent, pressure against the side of the can. The excess ice cream was always removed by a twisting motion against the side of the can. When an air hole in the disherful was attempted to be filled, an extra heavy disherful always resulted. It was also noticed that a ball showing an air space was not necessarily lighter than one which had a perfect semi-round shape.

The average data obtained when using the semi-round dishers is given in table 1. It will be noted from this table that the volume shrinkage is practically the same for all sizes of dishers.

TABLE 1

Effect of size of disher

SIZE OF DISHER	CAN			DISHERFULS		EQUIVALENTS		VOLUME PER CENT SHRINKAGE
	Number dished	Net weight	Overrun	Number	Average weight	Quarts from can	Dishers from a quart	
		pounds	*per cent*		*ounces*			
No. 10	4	13.7	97	70	3.1	7.0	5.8	42
No. 12	12	14.0	99	81	2.6	6.9	6.9	44
No. 16	4	14.2	93	117	1.9	7.4	9.8	36
No. 20	3	14.0	93	131	1.7	6.8	10.9	44
No. 24	6	14.1	97	170	1.3	7.0	14.1	41
No. 30	3	13.9	98	209	1.0	6.9	17.1	42
No. 10	*1*	*13.6*	*105*	*94*	*2.0*	*9.4*	*7.8*	*22*
No. 20	*1*	*13.2*	*110*	*167*	*1.3*	*8.3*	*13.8*	*31*

This may be explained in that the larger scoops require more pressure to fill them, where-upon the air is squeezed out to a certain degree, and while the smaller scoops require less pressure per scoopful, nevertheless they require this smaller pressure to be applied to a smaller volume of ice cream, and also this smaller pressure must be applied more often or more times per each can dished. Therefore the effect of the smaller pressure is more additive and equals the larger pressure when the whole can is considered.

In order to obtain data which might show the effect of heaping the scoop, two cans were dished and each disherful leveled off

with a spatula. The results are added at the foot of table 1 and are indicated by italics. These figures are of interest because the variations in the sizes of the disherfuls should have been nil. They also show that a considerable part of the shrinkage was due to the heaping of the scoops. This factor will be considered again later when the results of loading the ice cream into cartons are given.

Two balls dished from the same can and using the same size scoop varied at times as much as 2 ounces in weight.

Averaging the weights of the disherfuls from the top third, the middle third and the lower third of the can, no difference was noted. This indicated that the ice cream remaining in the can was not compressed by the dishing out of that above it. This held throughout the entire work. That this condition would not have held with all operators is fairly certain.

When other tools than the dishers were used for dishing, the ice cream had to be loaded into cartons in order to have any figures relative to shrinkage. The pint tapered carton was arbitrarily selected for a standard.

The ice cream spoon had a $2\frac{3}{4}$ by $3\frac{1}{2}$ inches bowl with a somewhat square end and a short wooden handle. The "trowel" was of aluminium, such as is used for leveling ice cream in slab moulds. The "trowel" was $4\frac{1}{2}$ inches long by 3 inches wide and had its outer end curved somewhat to fit the curvature of the can. The "round loader" was one now on the market which cuts a cylindrical cake of ice cream from the can. The "square loader" was also one now on the market which cuts a square cake from the can. Both of these loaders are well known to the trade.

In certain instances the shrinkage when computed from the number of scoops obtained from a can was more than was indicated when this same ice cream was placed in the cartons. It may at first seem strange that the shrinkage could decrease upon further handling. But, as may readily be concluded from a careful consideration of the conditions, this decrease in shrinkage should have been expected because the ice cream balls when placed in the carton do not fit evenly and exactly together.

The air spaces remaining account for the increase in volume. And some of these air spaces are bound to remain, regardless of the pressure used in pushing the ice cream into the container. Ice cream once compressed would resist more strenuously any further compression. Many of the cartons were stripped in order to observe the number and sizes of these air spaces. This was done with cartons filled by all the tools. Aim was made to fill all cartons to a degree of firmness that would satisfy the retail trade. Consistent filling was at all times a chief objective. Care was likewise expended that the work did not become fussy to the extent that it would not approximate trade conditions.

TABLE 2

Effect of type of tool

TOOLS	CAN			PINT TAPERED CARTONS		VOLUME PER CENT SHRINKAGE
	Number	Net weight	Overrun	Number from can	Average weight	
		pounds	*per cent*		*ounces*	
Disher all sizes, average	9	14.0	95	14.1	15.2	39
Spoon	7	13.9	96	16.6	12.9	30
Trowel	5	14.0	94	17.3	12.7	28
Round loader	3	14.5	91	20.0	10.5	16.7
Square loader					9.9	

The weight of the pints filled by the dishers was influenced a great deal by the manner in which the first few balls were placed into the carton. The bottom of the carton being narrower, the balls did not fit together as snugly. Successive balls were placed in alternate sides of the carton. Table 2 gives the results when different types of tools were used. Employing the ice cream spoon as the dishing tool, less shrinkage resulted than when the dishers were used. In dishing with the ladle, care was exercised not to take too large ladlefuls. If they were too large, more pressure was required to push the ice cream into the carton and the less likely were the air spaces to be filled, even though the greater pressure was applied. Also if too small a spoonful was taken each time, the ice cream received more intensive handling and therefore lost more of its overrun.

Most of the cartons were carefully examined for air spaces. This was accomplished by submerging the carton in hot water for a moment and then inverting it over a paper plate, whereupon the ice cream fell out intact as a cake and was easily observed. All the cakes so examined were found to be free from air holes which were larger than would be tolerated by the most exacting customer.

In the use of the trowel the same general procedure was followed and the same factors were involved as applied to dishing by use of the spoon. With the trowel, however, it was easier to cut a cake of ice cream which more nearly fitted into the carton. This enabled the operator to dish ice cream into the cartons and have the ice cream retain more nearly the properties it had when in the can. A special case in which the carton is of the round type, will be given later when the effect of the type of carton is considered. The trowel and round carton were the best combination.

For filling the tapered cartons, the trowel should be wide as the top of the carton. This allowed a better filling of the carton for as the ice cream seated itself, it conformed snugly to the shape of the carton and filled the corners nicely without having to be pushed and crowded by excessive pressure.

Very little success was obtained from use of any of the semi-mechanical loaders, so designed as to cut a cake of ice cream to fit exactly into the particular carton for which the loader was adapted.

The loaders cut the ice cream from the can without altering the condition of the ice cream in any way. Thus the lightest weight pints were obtained by use of these dishers which dished the ice cream with very little shrinkage. However the author believes the objections to their use offset the advantages. All such dishers needed to be dipped into water after each time used. This was messy at best. The wet tool dripped water into the can which water would later freeze and give a coarse or lumpy texture to the ice cream subsequently dished. These tools having a large surface area, considerable water was therefore carried by them. Also considerable ice cream was carried to

the wash water. So, after two or three usages, the water became quite dirty. When this water dripped upon floor and cabinet the messy condition constantly increased. The wash water being relatively warm at best, would offer a good place for the culture of bacteria which would eventually be carried to the ice cream.

These loaders were necessarily of small enough dimensions to allow of their introduction into the container before the expulsion of the ice cream. Consequently the cake of ice cream was not as wide as the carton and an air space was left between the ice cream and the carton. Although the disher did dish a true pint, nevertheless the carton of ice cream did not look full and actually the ice cream did not measure a full pint if much pressure was used in expelling it. If the tool was allowed to get too cold, the pressure necessary for ejection became very great.

With the round type of tool, the ice cream cake could be broken off at the bottom from that remaining in the can by a twisting motion. However with the square instrument this could not be accomplished as easily. Only with intense rocking could the cake be freed from the remaining ice cream. This procedure necessarily squeezed and compressed the ice cream adjacent to the loader in position.

The temperature of the loaders needed careful regulation which was impractical. If too cold, ice cream stuck to the outside and great pressure was needed to expel the cake. If too warm, the cake often would not break at the bottom.

The data for all cartons filled showed that there was no perceptible increase in weight of the cartons filled from the bottom of the can over those filled from the top. Thus it would seem that there was no compression of the ice cream when the upper portions were dished.

B. Effect of overrun

The relation of overrun or per cent of swell would seem of utmost importance in a study of shrinkage from dishing manipulations because the shrinkage in volume is due to the squeezing out of some of the air incorporated into the ice cream during the

freezing process. One would naturally infer that the larger amount of air so incorporated would result in a greater shrinkage when that particular ice cream was dished. That the results of this investigation do not uphold such a conjecture may seem rather startling at first but when the factors involved are carefully studied, the data here cited would be expected.

From the data in tables 3 and 4 it is readily seen that the overrun has hardly any effect upon the number of pints obtained, namely the shrinkage, but it does, however, govern their weight.

TABLE 3

Influence of overrun on number of disherfuls

TOOL	CAN			DISHERFULS		EQUIVALENTS		VOLUME PER CENT SHRINKAGE
	Number dished	Net weight	Overrun	Number	Average weight	Quarts from can	Dishers from quart	
		pounds	*per cent*		*ounces*			
Disher No. 10	4	13.7	97	70	3.1	7.0	5.8	42
	2	13.1	112	78	2.8	7.3	6.5	34
Disher No. 12	12	14.0	99	81	2.6	6.9	6.9	44
	4	15.2	80	84	2.8	7.0	7.0	41
Disher No. 16	4	14.2	93	117	1.9	7.4	9.8	36
	1	15.2	77	131	1.89	8.2	10.9	32
Disher No. 20	3	14.0	93	131	1.7	6.8	10.9	44
	1	13.3	107	133	1.58	6.7	11.1	44

This fact is in accord with the findings of Ruehe (1) but is not substantiated by the work of Fisher and Judkins (2), or by Bierman (5). It is of interest that one of these investigators dished at this laboratory 18⅛ pints from a can of 85 per cent overrun and 16⅛ pints from a can of 125 per cent overrun, both cans containing ice cream from the same mix.

The fact that the overrun was found to play such a minor rôle as regards shrinkage may be explained in that the greater the percentage of overrun, the more pliable the ice cream became,

and less pressure was required to fill the dishing utensil and the carton. There was slightly more shrinkage in cans of very high overrun as compared with cans of very low overrun. There was probably a difference of about 1 pint at most in amount of ice cream dished for overruns between 60 per cent and 120 per

TABLE 4

Influence of overrun on number of pint cartons filled

TOOL	CAN			PINTS OBTAINED		VOLUME PER CENT SHRINKAGE	TYPE CARTON USED
	Number	Net weight	Overrun	Number	Average weight		
		pounds	*per cent*		*ounces*		
All Dishers	9	14.0	95	14.1	15.2	39	Pint tapered
No. 12	E241	13.7	102	14.0	15.6	42	Quart tapered
	F333	14.9	81	14.0	17.0	42	Quart tapered
	F343	13.3	103	13.0	15.6	46	½ pint tapered
	F321	14.8	82	14.0	16.8	42	½ pint tapered
Spoon	7	13.9	96	16.6	12.9	30	Pint tapered
	C142	12.7	112	18.0	10.8	25	Pint tapered
	C121	13.2	105	19.0	11.2	24	Pint tapered
	B101	15.9	75	18.0	14.2	25	Pint tapered
	E312	15.6	77	18.0	14.1	25	Pint tapered
Trowel	5	14.0	94	17.3	12.7	28	Pint tapered
	F372	12.1	120	17.0	11.0	29	Pint tapered
	E303	13.4	106	16.0	13.2	33	Pint tapered
	F352	14.9	81	17.0	14.2	29	Pint tapered
	F323	16.1	67	17.0	14.7	29	Pint tapered
	F373	12.3	120	20.0	9.6	17	Pint round
	E282	14.6	90	20.0	11.5	17	Pint round

cent, a range of 60 per cent in overrun. For any ordinary ranges in overrun the shrinkage differences were negligible.

Again emphasis is put upon the fact that all the work was done in as uniform and constant manner as was possible in order that the effect of the overrun and of that one factor alone might be studied. In actual dishing by the average retailer he would find the high overrun would compress easier and therefore give

it more severe treatment than he would to ice cream of harder consistency.

C. A study of personal equation

When the results of work done by the author on the influence of overrun were reviewed, it was decided to have cans of varying overrun dished by different operators. The results of this phase of the investigation are listed in table 5. It is of interest to

TABLE 5

Results of different operators

OPERATOR	OVERRUN	TAPERED PINTS	AVERAGE WEIGHT	REMARKS
			ounces	
A	95	18.0	11.9	Good looking packages
	125	18.5	10.1	
B	95	19.0	11.5	Too careful; long time; small spoonfuls
	125	17.6	11.0	
C	95	15.0	14.1	Pressed too hard
	125	15.5	12.4	
D	85	18.3	12.6	Difference of 40 per cent in swell
	125	16.8	10.8	
E	90	18.0	13.1	Very poor; air spaces
	125	13.5	14.5	
F	67	17.0	14.7	Typical for author; difference of 53 per cent in swell
	120	17.0	11.0	

Average difference low and high overrun = 1.1 pints more from low.

note that the average results are consistent with those obtained by the author. The same operator, even when experienced, will very likely get a difference of one pint or even more when dishing in like manner two cans containing identically the same ice cream. This will account for the occasional slight variation from dishing cans of ice cream from the same mix and batch and which have had exactly the same treatment.

D. Size and shape of carton

In order to study all factors encountered in actual trade conditions, experiments were conducted in which the ice cream was dished into cartons of varying sizes and of various styles.

As is shown by table 6, the size of the carton does not influence the shrinkage except to very slight degree. In general the smaller the size of the carton, the greater was the shrinkage observed in dishing yet this difference was not very great,

TABLE 6

Influence of size of carton

TOOL	CAN			CARTONS FILLED			EQUIVALENT		VOLUME PER CENT SHRINKAGE
	Number dished	Net weight	Overrun	Size	Number	Weight	Number of pints	Weight of pint	
						OUNCES			
Disher No. 12	9	14.0	95	Pints	14.1	15.2	14.1	15.2	39
	3	13.8	95	Quart	7	32.0	14.0	16.0	42
	3	13.9	96	½ pint	27	7.9	15.5	13.8	44
Spoon	7	13.9	96	Pints	16.6	12.9	16.6	12.9	30
	2	13.8	95	Quart	8.5	25.9	17.0	12.9	29
	3	14.6	88	½ pint	33	6.6	17.3	13.2	28
Trowel	5	14.0	94	Pints	17.3	12.7	17.3	12.7	28
	2	14.2	95	Quart	8.5	26.3	17.0	13.2	29

amounting on the average to about one-half pint per can between quart and one-half pint size cartons.

The lack of any marked variation in shrinkage due to the size of carton used together with the uniform shrinkage noted when the disherfuls were and were not placed in cartons seems to indicate that the most of the shrinkage was due to getting the ice cream out of the can and not due very greatly to the process of packing it into the carton. The differences which were observed were probably due to the tendency of the larger cartons to bulge at the sides. Also it may have been due somewhat to

the fact that the disherfuls fitted in different manner into the different sizes of cartons.

The results of work using the round and the tapered carton which are listed in table 7 showed that there was practically no difference in the effect of the type of carton upon shrinkage except in the special case when the trowel and the round carton were employed. This was due to manipulation. The trowel was used to cut a large cake of ice cream from the can. Then using the round carton as a sort of cookie cutter, it was pressed down

TABLE 7

Influence of type of carton

TOOL	CAN			PINT CARTON			VOLUME PER CENT SHRINKAGE
	Number dished	Net weight	Overrun	Style	Number	Weight	
		pounds				*ounces*	
Disher No. 12	9	14.0	95	Tapered	14.1	15.2	39
	3	13.7	97	Round	14.6	14.8	39
Spoon	7	13.9	96	Tapered	16.6	12.9	30
	4	13.5	98	Round	16.9	12.8	30
Trowel	5	14.0	94	Tapered	17.3	12.7	28
	3	13.7	100	Round	20.0	10.7	17

Note effect of trowel with round carton!

over the ice cream held on the trowel. In this way a cake of ice cream was cut out which just fitted into the round carton. The sides of the round carton being stiff, this procedure was an easy one. Two such cuts usually filled the round carton very nicely and by twisting at the end of the second cut, a nice smooth surface, flush with the edge of the carton was obtained. Thus it was found possible to dish the ice cream with very little shrinkage when this combination of tools was used. This furnishes an ideal method for dishing ice cream.

E. Effect of dishing temperature

Conditions surrounding the retailing of ice cream vary greatly but probably the greatest variable factor is the temperature of the cabinet. This has been in the past particularly true although the widespread introduction of the mechanically refrigerated cabinets and other improved types, have done much to remedy the poor temperature conditions, still there is great interest in the proper temperature for dishing and the relation between temperature and shrinkage.

During the early work of obtaining the number of disherfuls from cans, when each disherful was weighed, it was noticed that the individual disherfuls became heavier if the ice cream became

TABLE 8

Effect of dishing temperature

TEMPERATURE	CAN			PINTS		VOLUME PER CENT SHRINKAGE
	Number dished	Net weight	Overrun	Number	Weight	
°F.		*pounds*			*ounces*	
5	3	14.0	92	17.1	13.0	29
10	8	13.9	96	16.5	13.0	30
15	3	13.8	96	16.6	13.1	31
23	1	13.7	96	20.0	9.88	17

very soft or if it became very hard. The soft ice cream evidently filled the disher more solidly while the hard ice cream required more pressure to get the disherful and therefore suffered loss of air. These results however did not effect the weights or numbers of pint cartons filled because in pushing the ice cream into the cartons, the very soft ice cream could not be subjected to any great pressure, it being squeezed out it attempted, while the very hard ice cream would not pack under even severe pressure and therefore air spaces were left between the lumps. This reasoning applies to all ice cream dished at differing temperatures. Therefore the temperature equation becomes practically zero except for very wide variations. There were two factors working against each other, the firmness of the ice cream and the

pressure necessary for proper dishing. These effects very nearly counterbalanced each other.

The data observed in this section of the research are given in table 8. The cans dished in this study were all tempered at the respective temperatures for at least twelve hours. All the dishing was done using the ice cream spoon and the pint tapered cartons. So it may be seen from this table that the temperature effect is practically zero except in very extreme cases.

The best dishing temperature was found to be 10°F.

F. Effect of varying composition

The next factor to be investigated was whether the composition of the mix might have some vital connection with the

TABLE 9

Mix I—14.0 per cent fat, 38 per cent total solids

TOOL	CAN			PINTS		VOLUME PER CENT SHRINKAGE
	Number dished	Net weight	Overrun	Number	Weight	
Disher No. 12	Average for standard mix from table 2					39
	3	pounds 13.7	96	14.1	ounces 15.2	41
Spoon	Average for standard mix from table 2					30
	3	13.8	97	17.3	12.7	28

dealer's shrinkage. There was no marked differences in shrinkage from ice creams made from mixers of varying viscosities and acidities.

For study of the influence of actual variation of composition, four special mixes of two hundred pounds each were prepared in which the percentages of fat, of total solids, and the amount of filter were varied one at a time and in which all other ingredients were kept standard. Mix IV contained no egg powder. All other mixes used in this research contained 0.5 per cent yellow egg powder.

As shown by data given in tables 9 and 10, the percentage of

TABLE 10

Mix II—8.0 per cent fat, 38 per cent total solids

TOOL	CAN			PINTS		VOLUME PER CENT SHRINKAGE
	Number dished	Net weight	Overrun	Number	Weight	
Disher No. 12	Average for standard mix from table 2					39
	3	*pounds* 14.3	93	13.6	*ounces* 15.8	43
Spoon	Average for standard mix from table 2					30
	3	14.4	93	16.1	14.2	32

TABLE 11

Mix III—34.0 per cent solids, 12 per cent fat

TOOL	CAN			PINTS		VOLUME PER CENT SHRINKAGE
	Number dished	Net weight	Overrun	Number	Weight	
Disher No. 12	Average for standard mix from table 2					39
	3	*pounds* 14.0	97	14.1	*ounces* 15.6	41
Spoon	Average for standard mix from table 2					30
	3	13.7	96	17.3	13.0	28

TABLE 12

Mix IV—no egg powder, 12 per cent fat, 38 per cent total solids

TOOL	CAN			PINTS		VOLUME PER CENT SHRINKAGE
	Number dished	Net weight	Overrun	Number	Weight	
Disher No. 12	Average for standard mix from table 2					39
	3	*pounds* 14.4	93	14.1	*ounces* 16.2	41
Ladle	Average for standard mix from table 2					30
	3	14.2	95	17.0	13.2	29

fat plays a very minor rôle in the shrinkage even when the per cent of fat is varied between 8 and 14 per cent a difference of 6 per cent. The limits of 8 and 14 per cent were chosen because most ice cream today on the market would come within these percentages.

Next the per cent of total solids was varied by decreasing this ingredient 4 per cent. The results for mix III containing 34 per cent solids are given in table 11.

Here again a variation in composition, this time in the per cent of total solids cannot be attributed to causing any very marked change in amount of shrinkage.

The action and value of filler has always been of interest and so the next mix studied was made up with no egg powder. The results for mix IV containing no egg powder are given in table 12.

Yet again there is no noticeable difference whether the composition was altered, this time the egg seeming to have no great influence on shrinkage.

It is the opinion of the author, however, that all variations in composition do influence the properties of the ice cream, and so also the shrinkage upon dishing. The effect would have to be very marked indeed to show when a three gallon can was dished. But it is evident that these influences are of a very minor character when considered with the other factors such as the operator.

SUMMARY

1. The shrinkage of ice cream when dished from bulk containers averaged about one-third of its original volume.

2. The shrinkage was nearly the same regardless of the size or type of disher or the size and shape of the carton used.

3. The ice cream spoon was found to be the most practical dishing tool.

4. The effect of the overrun on the volume shrinkage was not very marked.

5. Method of dishing and filling of the cartons was ascribed as being of vital significance on the shrinkage encountered in commercial practices.

6. The effect of the dishing temperature was not very great unless extreme temperatures were encountered.

7. The composition of the mix did not influence the volume shrinkage sufficiently to be of importance when compared with item 5.

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A STATISTICAL STUDY OF CREAMERY OPERATION*

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Conclusions drawn from general observations by the persons engaged in an industry are not always true. Fortunately, the statisticians have developed methods of analyzing large numbers of figures so that it is possible to weigh fairly accurately the facts that a certain set of data contains.

The data on which this article is based were obtained by survey[1] from 78 creameries in Minnesota for the year 1919. All of these creameries were of the farmer type, such as is found in villages and in the smaller cities. None were centralizers. There were 71 farmers' coöperative plants, and 7 independent or proprietary plants.

There was a wide variation among these 78 creameries. Some were small and a few were large. Some were housed in old, worn-out frame buildings, but the majority were operating in new homes, most of which were constructed of brick. Some of the creameries were conveniently arranged, and a few were very inconvenient. Some had good accounting systems, and others had very poor records. Most of the creameries were efficiently administrated, but a few apparently were not managed at all,

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† The author wishes to acknowledge the suggestions of Dr. F. A. Pearson, of the Department of Agricultural Economics and Farm Management at Cornell University, in assembling the data.

[1] The survey was made by the writer, who represented the Federal Bureau of Markets and who collaborated with Dr. John D. Black, Chief of the Division of Agricultural Economics of the University of Minnesota. In all, 102 creameries in 42 counties were visited. Some of the reports were not sufficiently complete to be used, and so only 88 creameries were included in the final analysis. The summary of that study was published in Technical Bulletin 26 of the University of Minnesota, 1924, entitled "Economic Aspects of Creamery Organization." The supply of that bulletin is now practically exhausted. The present article is a second study of some of the same data, but from a different angle. Certain data are missing in a few of the reports of the 88 creameries considered in the first study, and hence only 78 plants are included in the present analysis.

they ran themselves. Some had sufficient capital on which to do business, and others were "hard up." Some received good cream and took pride in making "higher scoring" butter, while others paid little attention to the quality of their product.

The relationships that existed between several of the important items in the manufacture of butter in these 78 creameries may be shown in different ways. The mean, the standard deviation, the coefficient of variability, the coefficient of correlation, and the probable error were calculated, and these all help to shed light on what actually took place.

The nine important factors in the manufacture of butter, which were studied, are:

x_1. Price paid for fat in cents. This is the price which the farmer received for his butterfat.

x_2. Price received for butter in cents. This is the price which the creamery received for the butter.

x_3. Score of butter. The writer scored the butter at the creamery. All of it was less than one week old.

x_4. Percentage overrun. Overrun is the increase in butter over the butterfat. Overrun may be defined as the sum of the casein, the salt, and the water in the butter, minus the losses in manufacturing. Good commercial creamery butter contains about 81 per cent of fat.

x_5. Cost of labor per hour, in cents. The value of the time of directors, secretary, treasurer, bookkeeper, manager, workmen in the plant, and workmen's compensation, is all included in this calculation.

x_6. Hours of labor to make 1000 pounds of butter. All of the labor within the creamery that went into the butter was recorded.

x_7. Pounds of butter made in 1919, in thousands. This is the volume, or amount, of butter manufactured in each creamery in 1919.

x_8. Building cost per 1000 pounds of butter, in cents. This item includes interest, maintenance, depreciation, taxes, and insurance, on the buildings. The annual net cost was divided by the annual production.

x_9. Equipment cost per 1000 pounds of butter, in cents. This factor includes interest, maintenance, depreciation, taxes, and

insurance, on the equipment. The annual net cost was divided by the annual production.

MEAN, STANDARD DEVIATION, VARIABILITY, AND PROBABLE ERROR

The means and the variables for the nine factors in this study are given in table 1. The mean, or average, price paid to the farmers for butterfat, was 64.8 cents, whereas the price received for the butter was 56.4 cents. On the average, therefore, the 78 creameries paid 8.4 cents more for the butterfat than they received for the butter.

TABLE 1

Mean, standard deviation, and coefficient of variability

	MEAN	STANDARD DEVIATION	COEFFICIENT OF VARIABILITY
1. Price paid for fat, in cents	64.75± 0.30	3.91± 0.21	6.04±0.33
2. Price received for butter, in cents	56.36± 0.16	2.07± 0.11	3.68±0.20
3. Score of butter	91.23± 0.12	1.51± 0.08	1.65±0.09
4. Percentage overrun	23.82± 0.14	1.83± 0.10	7.70±0.42
5. Cost of labor per hour, in cents	44.38± 0.84	11.00± 0.59	24.78±1.42
6. Hours of labor to make 1000 pounds pounds of butter	35.59± 1.15	15.13± 0.82	42.51±2.68
7. Pounds of butter made in 1919, in thousands	230.21±11.64	152.39± 8.23	66.19±4.89
8. Building cost per 1000 pounds of butter, in cents	603.56±25.85	338.53±18.28	56.08±3.86
9. Equipment cost per 1000 pounds of butter, in cents	420.67±13.83	181.08± 9.78	43.04±2.72

The average score was 91.2, which was fairly well toward "extras," the range being from 87 to 94.

The average overrun was 23.82 per cent, which is high. In passing, it should be stated that the high overrun obtained by many creameries was due to the practice of not weighing the half pounds. For illustration, if a can of cream weighed 35½ pounds the creamery would record it as 35 pounds. Thus the half pounds were not counted in the volume of butterfat, but they were included in the weight of the butter. It is apparent that the overrun would be increased thereby.

The average cost of labor in 1919 was 44.4 cents per hour, with a range from 26 to 80 cents. The hours of labor to make 1000 pounds of butter showed an average of 35.6, with a range from 16.1 to 85.2 hours. The volume of butter, or the amount manufactured was 230,218 pounds and ranged from 31,000 to 886,000 pounds. The average building cost was $6.04 per thousand pounds of butter manufactured, and the range was from $1.41 to $7.80. The equipment cost per thousand pounds of butter made was $4.21, with a range from 82 cents to $9.17.

One of the striking features of the study is the variability shown.[2] Statisticians have constructed an index of variability which, to speak figuratively, is nothing more than a thermometer showing how things vary. It is like "percentage" in that it quickly and clearly makes a comparison discernible; it also measures the difference in variation. The variation in score of butter is small, due to the limited range of butter scores. Butter scoring 86 is very poor, while a score of 94 indicates a very high grade of butter. The coefficient of variability for "score of butter" was 1.7. The most variable factor was "pounds of butter made in 1919, in thousands," the coefficient of which was 66.2.

The variability coefficient of the price paid for butterfat was 3.68, which was about twice that of the price received for the butter. The price which the creamery gets for butter is based on the market conditions and the grade of the butter, and, as has already been pointed out, the score of butter is very non-variable. The price which the farmer receives for the butterfat is more variable, because, in addition to the price of the butter, it is affected by many of the problems of manufacture, particularly the hours of labor consumed in making the butter, and the volume of output.

The cost of labor is about four times more variable than the price of fat. It is affected more by volume than by any other factor, although the hours of labor required to make a unit of butter is directly related.

[2] Coefficient of variability $= \frac{\sigma}{Me} 100.$

CORRELATION

The explanation of the significance of the coefficient of correlation is very aptly expressed as follows by G. F. Warren[3] when he says:

> A coefficient of correlation furnishes a convenient means of expressing the relationship of one set of facts to another set. For example, the daughters of good cows tend to be, but are by no means sure to be, good cows. If it were an absolute certainty that every daughter of a cow would produce precisely the same amount of butterfat as her mother produced, the correlation would be expressed by 1. If there were not the slightest relationship between the amount of butterfat given by a cow and that given by her daughters, the correlation would be 0. Studies of butter production have shown that the correlation is 0.28. This shows that there is a considerable chance that the daughter of a cow that produced a large amount of butter will be a high producer, but that the results are far from certain. The relation of the heights of men to the heights of their sons shows a correlation of 0.51. . . . .
>
> Even the low correlation of 0.28 between the production of cows and that of their daughters is enough so that men are willing to pay more for the daughters of good cows than for daughters of poor cows.

The eight other variables were correlated with the price which the farmer received for the butterfat and with one another. The first line in table 2 shows the correlation coefficients between eight factors and the "price paid for butterfat." Likewise the second line correlates the seven factors with "price received for butter." In a similar way the coefficients of correlation given in the columns show the relationships between the column headings and the heading of the line on which the coefficients are located.

The correlation between the price received by the farmer for the butterfat, and the price obtained by the creamery for the butter, is very high ($r = 0.743$). The correlation between the price received by the farmer for the butterfat, and the score of the butter, also is high ($r = 0.631$). Overrun, on the other hand,

[3] Prices of Farm Products in New York. Cornell University Agr. Exp. Sta. Bul. 416, 1923, p. 33.

TABLE 2

Correlations between nine factors in seventy-eight creameries in Minnesota, in 1919

	(1) PRICE PAID FOR FAT, IN CENTS	(2) PRICE RECEIVED FOR BUTTER, IN CENTS	(3) SCORE OF BUTTER	(4) PERCENTAGE OVERRUN	(5) COST OF LABOR PER HOUR, IN CENTS	(6) HOURS OF LABOR TO MAKE 1000 POUNDS OF BUTTER	(7) POUNDS OF BUTTER MADE IN 1919, IN THOUSANDS	(8) BUILDING COST PER 1000 POUNDS OF BUTTER, IN CENTS	(9) EQUIPMENT COST PER 1000 POUNDS OF BUTTER, IN CENTS
1. Price paid for fat, in cents		0.743±0.03421	0.631±0.04506	0.196±0.07343	0.008±0.07636	−0.536±0.05442	0.407±0.06371	−0.228±0.07239	−0.233±0.07222
2. Price received for butter, in cents			0.600±0.04887	0.101±0.07559	0.068±0.07601	−0.343±0.06738	−0.104±0.07554	−0.129±0.07509	−0.153±0.07458
3. Score of butter				−0.016±0.07635	0.123±0.07521	−0.378±0.06543	0.291±0.06990	−0.053±0.07615	0.103±0.07565
4. Percentage overrun					0.049±0.07618	−0.037±0.07626	0.143±0.07480	−0.110±0.07544	−0.050±0.07617
5. Cost of labor per hour, in cents						−0.370±0.06591	−0.476±0.05906	−0.104±0.07630	0.034±0.07628
6. Hours of labor to make 1000 pounds of butter							−0.436±0.06185	0.418±0.07066	0.406±0.06378
7. Pounds of butter made in 1919, in thousands								−0.309±0.06907	0.246±0.07174
8. Building cost per 1000 pounds of butter, in cents									−0.241±0.07193
9. Equipment cost per 1000 pounds of butter, in cents									

When studying this table the reader should observe that the correlation is between the various readings on a line with the one at the head of the line. The four most significant columns are 2, 3, 6, and 7. There are, however, a few important correlations in columns 8 and 9.

shows a coefficient of only 0.196, in relation to this factor, which indicates that overrun in these creameries is not nearly so important a factor in affecting the price of butterfat as is the price of butter. There is one consideration in evaluating the significance of overrun, however, that should not be overlooked. If there was a greater variation in the percentage of overrun, the correlation would be higher. If the variations in overrun were from 10 to 24 per cent, the higher figures would have a greater bearing on the price of the butterfat than when the range is only from 18 to 26 per cent. Inasmuch as overrun is more uniform than a few of the other factors, it is of less importance.

The "price received for butter," "score of butter," "hours of labor to make 1000 pounds of butter," and "pounds of butter made in 1919, in thousands," have more to do with the price that the farmer receives for butterfat than do the other factors. It is also noticeable that "score of butter" has greater significance in relationship to "price received for butter" than does any other factor. Volume, or "pounds of butter made in 1919, in thousands," has a very low correlation with "price received for butter," whereas it was very significant when compared with the "price paid for fat."

About one-half of the correlations are positive and the other half are negative. However, if the correlation is 0.2 or 0.3 or more, it is significant regardless of whether it is positive or negative. The results of positive or negative correlations may be interpreted as follows. The coefficient of correlation of "price received for butter" and "price paid for fat" is 0.743, which means that the creamery which obtained a high price for its butter, paid a high price for the butterfat. Again, the correlation of "pounds of butter made in 1919, in thousands," to "cost of labor per hour, in cents," is –0.476, which shows that as the volume of butter manufactured declines, the cost of labor increases. It also shows a very significant relationship.

The "hours of labor to make 1000 pounds of butter" is highly correlated with the other items. On the other hand, the "cost of labor per hour, in cents," is of little importance. The probable explanation for so little correlation between these last two items

is the same as in the case of the overrun. The cost of labor per hour is about the same throughout the state, consequently the low correlation. But there is a wide range of hours in "hours of labor to make 1000 pounds of butter," and therefore a significant correlation would be expected.

Under "building cost per 1000 pounds of butter, in cents," and "equipment cost per 1000 pounds of butter, in cents," the correlation coefficients are low with the exception of the correlations existing between them and "hours of labor to make 1000 pounds of butter." This indicates that as the cost of the building and that of the equipment go up, the hours of labor increase. A wide range in these two factors may be observed in table 3, which may account in part for the high coefficients. Another reason for high correlation coefficients may be in the large investments in building and equipment in creameries where the plant was not operated at full capacity.

MULTIPLE CORRELATION

A multiple correlation was calculated for the following four variables: "Price paid for fat in cents," "price received for butter in cents," "score of butter," and "hours of labor to make 1000 pounds of butter."

$$R_{1.236} = 0.82$$

It is apparent that the multiple correlation between these most significant variables is higher than any of the gross correlations. This indicates that the combined effect of the three independent variables is greater than any one.

It seems apparent that the amount of labor which goes into butter, and the volume of output, together with quality (which is measured by the score and reflected by the price of the butter), are the important considerations in making butter.

TABLE 3

Original data for nine variables for 78 Minnesota creameries, 1919

NUMBER OF CREAMERY	PRICE PAID FOR FAT, IN CENTS	PRICE RECEIVED FOR BUTTER, IN CENTS	SCORE OF BUTTER	PERCENTAGE OVERRUN	COST OF LABOR PER HOUR, IN CENTS	HOURS OF LABOR TO MAKE 1000 POUNDS OF BUTTER	POUNDS OF BUTTER MADE IN 1919, IN THOUSANDS	BUILDING COST PER 1000 POUNDS OF BUTTER, IN CENTS	EQUIPMENT COST PER 1000 POUNDS OF BUTTER, IN CENTS
	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9
1	69.5	58.7	92	20.6	40.3	23.8	238	354	284
3	63.3	55.1	91	24.2	37.2	29.9	140	590	513
4	67.4	55.1	91	24.0	40.2	39.1	282	770	325
6	69.5	59.5	94	23.7	32.8	24.3	768	448	237
7	63.7	55.0	89	26.3	57.9	23.7	196	369	319
8	70.3	58.4	92	25.5	30.7	37.2	222	575	317
10	56.0	52.1	88	25.0	52.3	69.6	41	669	82
11	67.9	59.9	92	21.9	34.6	39.3	283	477	639
12	67.5	57.2	92	22.9	58.9	34.7	113	990	420
13	67.2	58.9	93	23.6	50.0	29.8	256	826	316
14	62.3	56.1	92	20.4	46.1	47.7	131	1780	807
15	68.5	58.5	93	26.0	64.7	28.2	225	776	483
16	68.0	58.7	92	26.4	46.8	34.0	170	1144	356
17	70.3	61.1	92	23.0	80.9	27.3	98	924	298
18	67.8	61.5	91	23.5	31.1	39.3	184	364	301
19	66.7	57.1	92	22.2	34.4	39.1	194	446	249
20	66.5	59.0	92	25.5	50.0	28.8	135	312	415
21	66.1	57.0	93	24.0	58.9	21.7	200	692	410
22	64.1	56.0	92	23.8	30.0	61.0	60	470	693
23	66.3	57.0	90	23.3	52.7	19.5	199	141	134
25	63.7	57.3	93	20.0	70.4	16.1	195	330	459
26	64.0	55.0	89	23.0	35.9	44.0	117	347	314
28	56.0	54.5	90	22.0	43.2	54.7	160	760	145
29	56.9	51.8	91	25.6	42.2	36.5	164	487	670
32	59.5	54.7	89	24.3	46.4	42.7	86	887	917
33	69.3	56.9	91	27.5	28.9	19.5	444	208	214
34	65.7	55.3	91	26.8	67.3	24.7	96	879	445
35	69.8	59.8	91.5	27.6	43.4	47.7	97	442	386
36	67.7	56.3	91.5	26.0	53.9	24.6	217	396	328
37	67.3	57.6	91	24.3	31.6	33.0	294	508	205
38	61.2	58.4	92	24.8	60.3	52.2	125	788	843
39	62.0	54.5	91	25.0	39.5	30.7	145	297	344
40	64.4	57.1	91.5	21.7	43.0	29.9	134	666	466
41	63.0	54.4	90	24.2	28.4	51.0	167	194	322
42	65.4	56.3	91.5	21.6	47.2	22.7	347	328	330
43	66.5	55.8	90	26.0	45.4	20.4	545	160	140
44	64.9	54.9	92	24.6	39.8	22.6	541	174	166
45	63.0	54.7	90	23.0	60.1	20.3	131	349	463

TABLE 3—*Continued*

NUMBER OF CREAMERY	PRICE PAID FOR FAT, IN CENTS	PRICE RECEIVED FOR BUTTER, IN CENTS	SCORE OF BUTTER	PERCENTAGE OVER-RUN	COST OF LABOR PER HOUR, IN CENTS	HOURS OF LABOR TO MAKE 1000 POUNDS OF BUTTER	POUNDS OF BUTTER MADE IN 1919, IN THOUSANDS	BUILDING COST PER 1000 POUNDS OF BUTTER, IN CENTS	EQUIPMENT COST PER 1000 POUNDS OF BUTTER, IN CENTS
	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9
46	63.7	56.6	89	25.2	42.5	37.9	492	460	384
47	64.2	55.1	91	24.3	40.1	32.3	198	219	335
48	62.0	55.3	91	23.1	51.4	34.0	106	1276	528
49	56.0	53.0	90	26.0	39.2	85.2	77	657	521
50	58.0	53.5	89	18.0	35.5	38.9	176	330	292
51	59.6	54.2	91	24.6	49.5	30.3	120	1029	380
52	67.8	58.5	92	23.5	27.6	40.0	209	433	320
53	65.8	55.6	91	24.5	26.2	46.9	209	530	469
54	67.1	55.8	92	24.7	44.7	26.8	246	428	425
55	68.0	57.1	92	24.0	49.7	17.8	443	454	349
56	67.0	55.2	91	24.0	41.1	33.0	197	876	588
57	68.2	58.8	93	25.2	38.7	23.1	464	543	456
58	68.7	57.3	93	23.2	42.5	26.5	559	252	290
59	64.3	56.9	92.5	25.0	42.7	27.8	256	538	464
60	58.1	55.2	90	22.7	44.3	72.1	62	1453	778
61	66.2	57.1	93	23.1	53.5	22.4	31	258	247
62	65.5	56.5	91.5	26.8	42.9	26.2	288	613	296
63	57.4	54.1	89	19.9	44.4	42.9	167	774	293
65	60.0	55.0	88	24.8	28.8	75.0	110	1515	621
68	64.1	55.4	91	22.5	28.5	34.0	236	928	492
69	68.5	56.9	92	22.7	40.2	23.1	263	319	694
70	63.0	54.5	91.5	24.0	57.0	24.2	147	286	659
71	68.8	54.3	92	22.8	38.3	78.5	136	1104	899
72	58.0	55.7	93	23.3	35.9	68.4	178	472	757
78	61.2	57.6	88	25.4	41.3	48.7	243	334	247
79	64.5	54.9	88	23.1	36.2	27.4	265	215	222
80	57.0	53.1	87	23.0	35.6	60.7	107	998	496
81	66.0	56.9	92	24.2	55.5	21.9	886	525	274
82	69.2	59.2	93	23.8	48.5	35.2	273	700	372
83	64.1	54.3	91	27.0	52.4	34.9	128	652	663
87	64.2	56.0	91	24.0	70.0	16.6	211	398	532
91	61.1	52.3	90	19.1	47.1	34.8	200	257	445
92	58.9	54.8	91	22.1	44.6	40.9	138	948	581
93	63.5	53.0	91	22.3	41.3	34.8	221	1400	292
94	69.3	58.6	94	24.0	55.3	20.2	341	487	297
96	68.8	56.3	92	24.7	39.7	25.4	482	799	399
97	68.5	58.8	94	23.2	37.4	33.0	200	603	478
98	67.8	58.2	92	22.1	44.0	26.8	207	833	331
99	70.2	59.1	94	22.8	27.1	36.8	339	573	376
101	67.5	58.2	91	25.5	43.3	27.9	206	292	516

TABLE 3—*Concluded*

For the student who desires to pursue the study further. The original data is presented in table 3. The sums of the nine variables, the sums of their squares, and the sums of their products are as follows:

$\Sigma X_1 = 5051.00$	$\Sigma X_1^2 = 328274.24$		
$\Sigma X_2 = 4396.10$	$\Sigma X_2^2 = 248099.01$	$\Sigma X_1X_2 = 285143.81$	
$\Sigma X_3 = 7116.50$	$\Sigma X_3^2 = 649464.75$	$\Sigma X_1X_3 = 461125.75$	$\Sigma X_2X_3 = 401231.90$
$\Sigma X_4 = 1858.00$	$\Sigma X_4^2 = 44523.10$	$\Sigma X_1X_4 = 120423.81$	$\Sigma X_2X_4 = 104749.04$
$\Sigma X_5 = 3461.90$	$\Sigma X_5^2 = 163061.95$	$\Sigma X_1X_5 = 224204.00$	$\Sigma X_2X_5 = 195232.84$
$\Sigma X_6 = 2776.50$	$\Sigma X_6^2 = 116688.53$	$\Sigma X_1X_6 = 177321.10$	$\Sigma X_2X_6 = 155642.54$
$\Sigma X_7 = 17957.00$	$\Sigma X_7^2 = 59455.82$	$\Sigma X_1X_7 = 1181768.10$	$\Sigma X_2X_7 = 100844.15$
$\Sigma X_8 = 47078.00$	$\Sigma X_8^2 = 373538.68$	$\Sigma X_1X_8 = 3025057.00$	$\Sigma X_2X_8 = 2646253.30$
$\Sigma X_9 = 32813.00$	$\Sigma X_9^2 = 16354309.00$	$\Sigma X_1X_9 = 2111972.90$	$\Sigma X_2X_9 = 1844878.50$
$\Sigma X_3X_4 = 169518.50$			
$\Sigma X_3X_5 = 316013.35$	$\Sigma X_4X_5 = 82542.30$		
$\Sigma X_3X_6 = 252645.85$	$\Sigma X_4X_6 = 66058.08$	$\Sigma X_5X_6 = 118419.20$	
$\Sigma X_3X_7 = 164356.00$	$\Sigma X_4X_7 = 430868.26$	$\Sigma X_5X_7 = 734699.00$	$\Sigma X_6X_7 = 560783.90$
$\Sigma X_3X_8 = 4293134.50$	$\Sigma X_4X_8 = 1116102.90$	$\Sigma X_5X_8 = 2059378.00$	$\Sigma X_6X_8 = 1842981.20$
$\Sigma X_3X_9 = 2995947.50$	$\Sigma X_4X_9 = 780318.60$	$\Sigma X_5X_9 = 1461664.60$	$\Sigma X_6X_9 = 1254757.60$
$\Sigma X_7X_8 = 9594909.00$			
$\Sigma X_7X_9 = 6760306.00$	$\Sigma X_8X_9 = 21846095.00$		
$M.X_1 = 64.7564$	$M.X_1^2 = 4208.6441$		
$M.X_2 = 56.3602$	$M.X_2^2 = 3180.7565$	$M.X_1X_2 = 3655.6898$	
$M.X_3 = 91.2371$	$M.X_3^2 = 8326.4711$	$M.X_1X_3 = 5911.8686$	$M.X_2X_3 = 5143.9987$
$M.X_4 = 23.8205$	$M.X_4^2 = 570.8089$	$M.X_1X_4 = 1543.9591$	$M.X_2X_4 = 1342.9364$
$M.X_5 = 44.3833$	$M.X_5^2 = 2090.5378$	$M.X_1X_5 = 2874.4102$	$M.X_2X_5 = 2502.9851$
$M.X_6 = 35.5961$	$M.X_6^2 = 1496.0068$	$M.X_1X_6 = 2273.3474$	$M.X_2X_6 = 1995.4172$
$M.X_7 = 230.2179$	$M.X_7^2 = 762.2541$	$M.X_1X_7 = 15150.8730$	$M.X_2X_7 = 1292.8737$
$M.X_8 = 603.5641$	$M.X_8^2 = 4788.9574$	$M.X_1X_8 = 38782.7820$	$M.X_2X_8 = 33926.3240$
$M.X_9 = 420.6794$	$M.X_9^2 = 209670.6282$	$M.X_1X_9 = 27076.5756$	$M.X_2X_9 = 23652.2884$
$M.X_3X_4 = 2173.3141$			
$M.X_3X_5 = 4051.4532$	$M.X_4X_5 = 1058.2346$		
$M.X_3X_6 = 3239.0416$	$M.X_4X_6 = 846.8984$	$M.X_5X_6 = 1518.1948$	
$M.X_3X_7 = 21071.2820$	$M.X_4X_7 = 5523.9520$	$M.X_5X_7 = 9419.2179$	$M.X_6X_7 = 7189.5372$
$M.X_3X_8 = 55040.1860$	$M.X_4X_8 = 14309.0110$	$M.X_5X_8 = 26402.2820$	$M.X_6X_8 = 23627.964$
$M.X_3X_9 = 38409.5833$	$M.X_4X_9 = 10004.0846$	$M.X_5X_9 = 18739.1615$	$M.X_6X_9 = 16086.6358$
$M.X_7X_8 = 123011.6500$			
$M.X_7X_9 = 86670.5897$	$M.X_8X_9 = 280078.1412$		

FURTHER INVESTIGATIONS WITH ADULTERATING SULPHURIC ACID SO AS TO INCREASE BABCOCK TEST READING*

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Two years ago the writer[1] reported on the possibility of adulterating sulfuric acid so as to increase the reading of the Babcock test in which such acid was used. That report was on a temporary emulsion of a fat solvent saturated with butterfat and thoroughly shaken with acid. This adulteration did not materially affect the appearance of the acid, but on standing for more than an hour, the fat saturated solvent would separate out and accumulate on top of the acid, in which condition duplicate tests would not check and consequently adulteration would be suspected.

Since that report, the writer has been able to secure an acid emulsion that is stable and that will increase the Babcock reading when such acid is used. One sample of such an emulsion made up on December 15 has stood without agitation, and on May 5 shows no signs of separation. This sample was made up of 400 cc. sulfuric acid, specific gravity 1.83, 16 cc. fat saturated benzine, and 6 cc. water soap solution (Palmolive china soap, 1 part, and water, 2 parts). As the soap water solution is not miscible with the benzine, it was added first to the cold acid and shaken when the saturated fat benzine solution was added and thoroughly shaken.

This acid was used in performing a Babcock test the following day without being agitated, when it gave an increased reading of 0.8. It was again used without agitation on February 15, when the necessary sample was carefully drawn by means of a

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[1] W. E. Petersen, JOURNAL OF DAIRY SCIENCE, vii, no. 4 (1924).

pipette from about the center, with the same results. Further tests on April 15 gave identical results.

Centrifuging this acid in an 18-inch centrifuge at about 10,000 revolutions per minute for ten minutes failed to cause separation. Examination under an oil emersion lens of 1200 magnifications failed to reveal any particles, indicating that in all probability the dispersion is colloidal and that the soap acts as a stabilizer.

The appearance of the adulterated acid would not arouse suspicion nor would its reactions with milk, in performing the Babcock test, as it behaves like normal acid. Separation of the adulterant is effected, however, by the addition of equal parts of water and centrifuging in usual Babcock test bottles in a Babcock centrifuge at the normal speeds for milking testing, by which means adulteration can be detected.

THE EFFECT OF THE PASTEURIZATION TEMPERATURE ON INDIVIDUAL GERMS FOUND IN MILK*

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Pasteurization, since its introduction into the dairy industry, has received much study. The primary reason for introducing this process into the dairy business was to protect the public from disease germs carried by milk and it is along this line that most of the study has been directed. Pasteurization also prolongs the keeping quality of the milk when it is properly done. It is stated that about 99 per cent reduction of the bacterial milk flora is obtained by this process. Ayers and Johnson (1) have published evidence showing that when milk is highly contaminated with bacteria a ninety-nine per cent reduction is secured, but when the milk is not so highly contaminated the percentage of reduction does not reach this figure.

The effect of pasteurization on the individual organism found in milk has not received much study. Ayers and Johnson (3) found that of twenty-two typical streptococci one survived a temperature of 62.8°C. for thirty minutes, and of 117 atypical streptococci, 38.46 per cent survived this temperature. The same authors (2) found twelve colon bacilli which resisted a temperature of 62.8°C. Barber (4), studying the effect of temperature on the rate of multiplication of a colon bacterium, found that it reached its maximum rate of division at 37°C. and that this rate fell off at 45°C. and ceased at 49°C. Tanner and Dubois (6) concluded from their work "that the members of the colon-typhoid group in milk in the number to which they would occur are destroyed by heating for thirty minutes at 60°C."

The purpose of this study was to determine the effect of pasteurization on pure cultures of bacteria found in milk. The

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† This short paper is to be considered as a preliminary report.

work was commenced June 15, 1925, and was concluded August 8, 1925. During this period seventy different types of bacteria were collected. These organisms were taken from platings made from unpasteurized milk, from rinsings made from dairy utensils and from the interior of the cow's udder. The organisms to be pasteurized were transferred to test tubes containing 5 cc. of sterile milk. The inoculated test tubes were incubated at 37°C. from two to five hours. Pasteurization was carried on in a copper jacketed water bath at a temperature of 62.5°C.

Just before pasteurization the milk was plated and then put into a water bath and pasteurized at a temperature of 62.5°C. for thirty-five minutes. The five minutes over the usual pasteurization time was to allow the milk to reach the above temperature. The milk was again plated immediately after pasteurization. The plates were incubated for ninety-six hours at room temperature and twenty-four hours at 37°C. If the plates made from pasteurized milk were not sterile, the organism was again inoculated into sterile milk, incubated and repasteurized using, instead of a test tube, a Sternberg bulb of about 5 cc. capacity. In some instances, it was found that milk would not be sterile when pasteurized in a test tube, but would be sterile when pasteurized in a Sternburg bulb. After the milk was pasteurized and plated, it was incubated for several days to observe whether the destruction of the germs was complete. An effort was made to not allow the germs to grow to such a number that their by-products would be a factor in their destruction during pasteurization.

All the organisms reported in table 1 produced acid in nutrient broth containing dextrose, and most of them produced acid in nutrient broth containing sucrose and lactose. None produced gas excepting the aerogenes like organism and the colon organisms.

There were forty-seven non-spore-forming organisms. Of these forty-seven, only two survived pasteurization. The two resisting organisms are 43 and 44. No. 44 is similar to, if not the same as, the organism reported by Russel and Hasting (5) several years ago to withstand a temperature of 76°C. It is a lemon color micrococcus. It produces acid in nutrient broth and grows rapidly in milk. Russel and Hastings report that their organism

TABLE 1

The effect of pasteurization on germs found in milk and dairy utensils

NUMBER OF ORGANISMS	UNPASTEURIZED	PASTEURIZED	REMARKS
		Non-spore formers	
1	36,000,000	Sterile	
2	3,420,000	Sterile	Ropy
3	1,790,000	Sterile	
4	7,660,000	Sterile	
5	16,140,000	Sterile	
6	32,400,000	Sterile	
7	43,200,000	Sterile	
8	115,000	Sterile	
9	7,300,000	Sterile	
10	166,800,000	Sterile	
11	61,200,000	Sterile	
12	28,900,000	Sterile	
13	1,620,000	Sterile	
14	1,020,000	Sterile	
15	651,000	Sterile	
16	54,600,000	Sterile	
17	11,000	Sterile	
18	23,500,000	Sterile	
19	27,000,000	Sterile	
20	1,185,000	Sterile	
21	59,400,000	Sterile	
22	114,000,000	Sterile	
23	14,500,000	Sterile	
24	330,000,000	Sterile	
25	109,200,000	Sterile	
26	112,900,000	Sterile	
27	2,100,000	Sterile	
28	114,000,000	Sterile	Gas forming. Aerogenes-like
29	78,000,000	Sterile	Somewhat similar to 28
30	5,100,000	Sterile	
31	21,800,000	Sterile	
32	1,500,000	Sterile	
33	3,400,000	Sterile	White colony, coccus, from U
34	6,500,000	Sterile	Yellow colony, coccus, from U
35	4,700,000	Sterile	Pink colony, coccus, from U
36	1,500,000	Sterile	
37	6,100,000	Sterile	Colon, human
38	20,000	Sterile	
39	27,000	Sterile	
41	30,600,000	Sterile	
42	40,200,000	Sterile	

TABLE 1—*Continued*

NUMBER OF ORGANISMS	UNPASTEURIZED	PASTEURIZED	REMARKS
		Non-spore formers—Continued	
43	36,000,000	1,020,000	
44	5,040,000	134,000	Micrococcus lemon color
45	10,000,000	Sterile	
46	15,000,000	Sterile	Streptococcus
47	2,100,000	Sterile	Aerogenes with reddish color
		Spore formers	
48	2,850,000	3,060,000	Brownish colony, rod forms spores so rapidly that it is hard to find vegetative organism
	600,000	460,000	
	4,600,000	2,390,000	
	2,250,000	5,050,000	
	4,800,000	9,600,000	
	2,500,000	4,040,000	
49	4,120,000	30,000	
50	640,000	33,000	
51	352,000	25,900	
52	165,000	96,000	
53	1,080,000	132,000	
54	6,700,000	300	Spores not numerous
55	13,000,000	2	Spores not numerous
56	84,000,000	500	Spores not numerous
57	1,200,000	100	Spores not numerous
58	21,000,000	10,810,000	
59	3,480,000	2,160,000	
60	800,000	780,000	
61	4,400,000	1,120,000	
62	1,600,000	149,000	
63	5,900,000	4,540	
64	180,000,000	7,200	
65	2,800,000	9,500	
66	72,000,000	1,000	
67	5,040,000	134,000	
68	1,560,000	1,140,000	
69	9,300,000	1,000	
70	14,600,000	1,020,000	

did not ferment dextrose, sucrose or lactose. No. 43 was not picked from the plates this year, but was secured from pasteurized milk two years ago and kept in the laboratory. At the time when it was found, it was causing counts of a million or more in pasteurized milk.

Most of the spore forming organisms reported here first coagulate milk and then digest it. But some of them digest the milk without coagulating it. No thermophils were found during the progress of this work.

TABLE 2

Effect of pasteurization on named organisms

NAME OF ORGANISMS	UNPASTEURIZED	PASTEURIZED
Bacillus viscosus	10,900,000	Sterile
Bacillus subtilus	5,200,000	32,100
Bacillus ramosus	2,400,000	24,000
Bacillus buytricus	4,200,000	266,000
Bacillus Glaligu	14,900,000	980
Proteus vulgaris	84,000,000	Sterile
Erythrobacillus ptodiosus	700,000	Sterile
Bacterium paracoli	7,200,000	Sterile
Bacterium communior	26,600,000	Sterile
Bacterium coli	9,400,000	Sterile
Bacterium aerogenes	8,800,000	Sterile
Bacterium acid lacti	144,000,000	Sterile
Bacterium aerogenes	2,000,000	Sterile
Bacterium alcaligenes	2,000,000	Sterile
Bacterium aerogenes soil	44,000,000	Sterile
Bacterium coli (bovine)	13,000,000	Sterile
Pseudomonus pyocyaneus	12,300,000	Sterile
Micrococcus albus	2,100,000	Sterile
Micrococcus Aurantiaca	144,000,000	Sterile
Staphylococcus aureus	7,900,000	Sterile
Micrococcus aureus	780,000	Sterile
Sarcina ventriculi	420,000	Sterile
Sarcina lutia	197,000	53,000
Micrococcus flavus	7,000,000	Sterile
Lactobacillus casei (epilson)	1,140,000	Sterile
Lactobacillus casei (alpha)	3,500,000	Sterile

Note: The authors accepted the names of the above organisms as they were on the tubes when received and no effort was made to verify them.

There was no instance where a spore forming organism was entirely eliminated by pasteurization. In two instances a larger count was obtained in the pasteurized milk than in the unpasteurized milk. This increase in number is apparently due to the breaking up of clumps of spores, since neither of these two organisms grew at temperatures above 50°C.

There was no effort made to determine the names of the organisms used in obtaining the data in table 1. The only criterion used was that the colonies were visibly different. In table 2 are given data on pasteurization of named organisms.

The outstanding point in table 2 is the same as in table 1, namely, that nearly all non-spore forming organisms were destroyed by pasteurization. Sarcina lutia is the exception in this case.

It will also be noted that the three colon organisms used in this work did not survive pasteurization. This is of interest because of the discussion as to whether all colon bacteria are destroyed by pasteurization.

Since only few of the organisms found in milk have been used in this work, no definite conclusion can be stated. More organisms were not used simply because they did not occur on the plates. This limited number may have been due to the fact that during the period of this experiment the weather conditions remained practically constant.

But the data presented indicate that a large number of the organisms found in milk do not survive a temperature of 62.5°C. Yet, in pasteurized milk, high counts are continually being found and these high counts are mostly due to non-spore-forming organisms. The reason for this is a problem for further investigation.

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A STUDY OF METHODS FOR BACTERIAL ANALYSES OF MARKET MILK*

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Bacterial counts of milk made upon different kinds of media, vary considerably in number present per cubic centimeter. This is due, largely, to the composition of the media used. As one kind of medium is most suitable for the growth of certain types of bacteria, it will give higher counts than other media when that particular type of organism predominates. If another type of organism predominates in the milk, another medium may be expected to give the higher counts.

It occasionally happens that a sample of milk with a count of 100,000 bacteria per cubic centimeter upon one medium will have a count of only half that number upon another medium. With other samples of milk the reverse may happen. Usually, however, certain media tend to give the highest counts, while other media tend to give the lowest counts.

The usual reason for making bacteriologicaal counts upon milk is to get an idea of how carefully the milk has been handled up to the time that the sample was taken. If the bacteriological count is low, we assume that the milk has been handled in such a careful way that good keeping quality and reasonable safety are likely. If the count is very high, we suspect that the milk has been so carelessly handled that good keeping qualities cannot be expected and safety from disease producing organisms is in doubt.

As a member of the committee upon methods of bacterial analyses of milk and milk products appointed by the International Association of Dairy and Milk Inspectors, I undertook in 1921 to show the value of plate counts obtained upon four kinds of

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† Professor Cooledge died in May, 1925. This paper was nearly ready for publication and is presented with some slight changes designed to clarify certain points which seemed a little obscure.—*G. L. A. Ruehle.*

agar commonly used in milk work and to show the relation of the counts to the pH score (1).

TABLE 1

Samples of milk in group I

		pH SCORE	BACTO AGAR	MEAT EXTRACT	MEAT INFUSION	AGAR A
1	Farmer 12	50	50,000,000	60,000,000	22,000,000	67,000,000
2	Same; a cool day	45	9,600,000	1,000,000	2,300,000	1,700,000
3	Farmer 22	50	600,000	160,000	250,000	800,000
4	Same; a cool day	95	150,000	30,000	50,000	100,000
5	Farmer 8	45	600,000	650,000	400,000	700,000
6	Same; a cool day	55	60,000	80,000	40,000	10,000
7	Farmer 25	45	100,000		200,000	
8	Same; a cool day	90	80,000	50,000	40,000	10,000
9	Farmer 13	50	400,000	200,000		
10	Same; a cool day	95	110,000	120,000	60,000	100,000
11	Farmer 21	45	5,000,000	6,000,000	12,000,000	8,000,000
12	Same; a cool day	55	1,500,000	1,000,000	1,200,000	2,500,000
13	Farmer 36	50	75,000	10,000	40,000	60,000
14	Same; a cool day	70	50,000	30,000	30,000	30,000
15	Farmer 24	50	700,000	180,000	360,000	
16	Same; a cool day	80	130,000	180,000	90,000	60,000
17	Farmer 20	45	1,000,000	1,500,000		
18	Same; a cool day	55	130,000	100,000	40,000	40,000
19	Farmer 54	50	100,000	700,000	700,000	800,000
20	Same; a cool day	55	45,000	80,000	40,000	20,000
21	Farmer 1	80	30,000	7,000	80,000	110,000
22	Same; a cool day	95	150,000	80,000	20,000	10,000
23	Farmer 6	50	120,000	210,000	180,000	300,000
24	Same; a cool day	90	10,000	50,000	20,000	10,000
25	Farmer 11	45	1,000,000	1,000,000	500,000	1,000,000
26	Same; a cool day	55	220,000	100,000	90,000	100,000
27	Farmer 5	45	1,000,000	12,000,000	40,000,000	15,000,000
28	Same; a cool day	90	90,000	40,000	20,000	140,000
29	Farmer 51	45	6,000,000	2,400,000		
30	Same; a cool day	90	10,000	30,000	10,000	10,000
31	Farmer 90	40	500,000	450,000	500,000	800,000
32	Farmer 50	80	370,000	50,000	90,000	100,000
33	Farmer 16	40	5,000,000	3,600,000		6,000,000
34	Farmer 2	50	3,300,000	3,100,000	4,000,000	5,000,000
35	Farmer 14	50	3,200,000	3,600,000	5,000,000	
36	Farmer 10	85	200,000	100,000	140,000	140,000

Early results of this work have been included in the committee reports (2), but my work has been continued and extended.

The methods used in this work are the 1921 Standard Methods for Bacteriological Examination of Milk, published by the American Public Health Association. Bacto agar used in groups I and II is out of lot 10445 which was furnished by the Digestive Ferments Company to members of the committee. Other Bacto agar was out of regular stock. Milk-powder agar was the milk-powder agar A described by Ayers and Mudge (3).

Media were adjusted to pH 6.5 to 6.8 where necessary. It was not necessary to adjust the reaction of Bacto agar and agar A.

Group I includes 36 samples of milk, 18 of which were taken upon a warm day and tested at once. The other 18 samples were taken from the same patron's cans a few days later, but upon a very cool day.

Group II includes 48 samples of milk, 24 of which were plated upon the four kinds of media and the pH score determined. The samples were then incubated at room temperature for two hours and retested.

Group III includes samples of milk plated upon different media and incubated at 37°C. for one hour and retested.[1]

It is fair to assume that the samples of milk in groups II and III when plated the second time should be in poorer condition bacteriologically than when they were tested the first time. If this is true, the method which shows the samples in poorer condition most consistently when tested the second time would be the better method.

Comparing the tests and retests on all of the samples of milk in group II and III using both the pH method and counts in various media, the sensitiveness of the methods appears as follows:

	Per cent efficient
pH score	91.0
Bacto agar	66.6
Meat infusion agar	66.6
Milk powder agar A	63.3
Beef extract agar	60.0

Greater sensitiveness of methods would be expected if the samples have a high initial bacterial count. Methods which show

[1] The detailed data for this could not be found.—*G. L. A. Ruehle.*

TABLE 2

Samples of milk in group II

		pH score	Bacto agar	Meat extract agar	Meat infusion agar	Agar "A"					Lactose infusion agar	Lactose extract agar
						Bacteria per cubic centimeter	Strong acid	Weak acid	Pepton-izers	Alk. and inert		
51	55	100	3,000	2,000	1,000	1,000				1,000		
52	55*	100	4,000	3,000	3,000	1,000				1,000		
53	6	100	5,000	7,000	8,000	8,000			1,000	7,000		
54	6*	95	7,000	1,000	1,000	2,000		1,000		1,000		
55	14	100	5,000	4,000	4,000	2,000				2,000		
56	14*	95	6,000	1,000	6,000	7,000				7,000		
57	21B	100	12,000	17,000	12,000	25,000		3,000	6,000	16,000		
58	21B*	85	14,000	9,000	7,000	16,000	2,000	4,000	1,000	9,000		
59	Raw, East Lansing Supply	100	9,000	10,000	15,000	15,000		10,000		5,000		
60	Raw, East Lansing Supply*	75	4,000	2,000	8,000	9,000		3,000		6,000		
61	19	95	10,000	10,000	10,000	10,000				10,000	10,000	10,000
62	19†	95	20,000	30,000	10,000	20,000				10,000	20,000	10,000
63	36A	95	10,000	10,000	10,000	10,000				10,000		
64	36A†	90+	10,000	20,000	30,000	40,000				40,000		
65	Pasteurized	95	10,000	10,000	10,000	90,000			10,000	80,000	20,000	10,000
66	Pasteurized†	90	20,000	50,000	10,000	40,000				40,000	20,000	10,000
76	13	95	20,000	20,000	30,000	10,000				10,000		
68	13†	90	10,000	10,000	10,000	40,000			10,000	30,000		
69	10	90	13,000	12,000	4,000	17,000			2,000		15,000	
70	10*	80	18,000	12,000	5,000	16,000	2,000			14,000		
71	24	80	20,000	10,000	30,000	20,000				20,000		
72	24†	80	10,000	30,000	100,000	40,000				40,000		
73	17	90	70,000	40,000	100,000	10,000				10,000	60,000	30,000

74	17†	75	60,000	20,000	20,000	40,000				40,000	50,000	90,000
75	50	85	60,000	80,000	90,000	110,000			20,000	90,000	50,000	120,000
76	50†	75	140,000	30,000	230,000	290,000				290,000	230,000	170,000
77	36B	85	12,000	10,000	12,000	19,000	6,000	12,000		1,000		
78	36B*	60	17,000	5,000	10,000	21,000		8,000		13,000		
79	23A	80	80,000	10,000	40,000	20,000				20,000		
80	23A†	80	60,000	30,000	60,000	60,000				6,000		
81	11	80	14,000	27,000	25,000	25,000	1,000	3,000	3,000	15,000		
82	11*	70	12,000	16,000	3,000	19,000		1,000	2,000	16,000		
83	23B	80	10,000	8,000	3,000	9,000				9,000		
84	23B*	55	18,000	3,000	7,000	7,000				7,000		
85	21A	75	160,000	250,000	270,000	900,000	50,000	50,000	10,000	790,000		
86	21A†	55	230,000	300,000	650,000	800,000	30,000			770,000		
87	8B	70	35,000	16,000	16,000	28,000	4,000	10,000	6,000	8,000		
88	8B*	50	150,000	117,000	580,000	250,000	20,000	100,000	5,000	125,000		
89	8A	65	20,000	10,000	20,000	50,000				50,000	90,000	10,000
90	8A†	55	80,000	30,000	30,000	50,000			20,000	30,000	400,000	70,000
91	16	55	2,100,000	1,200,000	700,000	900,000				900,000	640,000	1,000,000
97	16†	50	3,500,00	4,000,000	5,000,000	5,500,000				5,500,000	4,500,000	4,600,000
93	20	55	90,000	50,000	90,000	80,000				80,000	80,000	
94	20†	50	60,000	70,000	110,000	40,000				40,000		
95	2A	50	1,800,000	1,400,000	1,300,000	1,400,000	150,000	50,000	500,000	700,000		
96	2A†	45	2,700,000	3,400,000	2,600,000	2,100,000	120,000		1,500,000	480,000		
97	2B	45	900,000	254,000	1,200,000	1,030,000	130,000	210,000	60,000	630,000		
98	2B*	45–	1,140,000	950,000	5,500,000	4,500,000	100,000	160,000	130,000	4,101,000		

* Two hours at room temperature.

† Two hours at room temperature; twenty-two hours in ice box.

poorer condition upon retest of samples with low initial counts should be the more valuable.

If only those samples of milk having a retest average count of under 25,000 bacteria per cubic centimeter are considered, the efficiency of the various methods and media is as follows:

	Per cent efficient
pH score	84.6
Bacto agar	61.5
Infusion agar	30.0
Agar A	30.0
Extract agar	23.0

Considering only those samples having a retest average count between twenty-five thousand and 100,000, the results are as follows:

	Per cent efficient
pH score	85.7
Infusion agar	85.7
Agar A	85.7
Extract agar	71.4
Bacto agar	28.5

The samples of milk having retest counts between 100,000 and 1,000,000 bacteria per cubic centimeter give the following results:

	Per cent efficient
pH score	100
Infusion agar	100
Bacto agar	80
Extract agar	80
Milk powder agar A	60

The samples of milk with retest counts of over 1,000,000 give results as follows:

	Per cent efficient
pH score	100
Bacto agar	100
Extract agar	100
Agar A	100
Infusion agar	87.5

The pH score of the samples of milk after incubation was at times the same as before, but it never indicated that the milk was

in better condition after the samples had been subjected to the short incubation period and retested. In three instances the pH score indicates that the milk was in no worse condition after incubation that it was before. These were samples of milk which were usually good by this method when tested the first time.

In certain instances the samples of milk tested with the different kinds of media before and after incubation gave results which indicated that they were as good after the incubation as before. The samples may be classified as shown in table 3.

It seems that after the treatment which the above samples received, efficient methods should show the samples in worse condition upon retest. A few of the samples which were in very good condition at the start might show no change upon retest, but

TABLE 3

Condition of milk samples upon retest after short incubation period

HOW TESTED	POORER	NO CHANGE	BETTER
Meat extract agar	20	1	12
Milk powder agar A	20	2	11
Bacto agar	21	1	11
Meat infusion agar	22	2	9
pH score	29	3	0

certainly a reliable method should not indicate that a third of the samples were in an improved condition.

Many investigations have been made to determine the most suitable medium for milk work. It has usually been concluded in these studies that the method giving the highest counts is most efficient. It is argued that the medium giving the highest counts comes closest to giving counts which represent all of the bacteria present in the milk. There is a failure to agree upon the medium giving the highest counts. The reasons for this failure are considered below.

A tabulation of the samples of milk in groups I, II, and III, with average bacterial counts below 10,000 per cubic centimeter when plated upon four kinds of media shows that Bacto agar gives the highest counts and the milk powder agar A is next, with meat

extract agar the lowest. When samples with 10,000 to 25,000 bacteria were studied the counts upon milk powder agar A are the highest, with meat extract agar next, and meat infusion agar giving the lowest counts.

If a preponderance of samples is present with low counts one investigator might be expected to find one medium giving the highest counts while another investigator studying a poorer grade

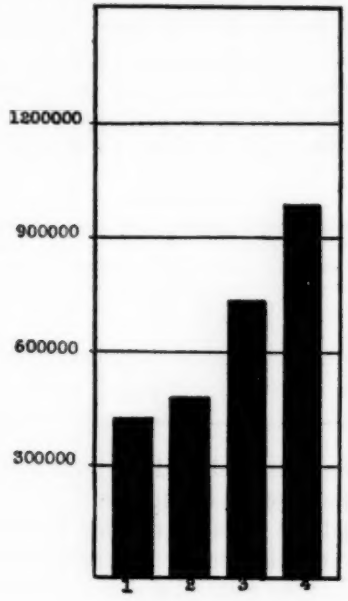

FIG. 1

of milk would find that another medium would give the highest counts.

The 102 samples of milk in groups I, II, and III gave average counts upon the four kinds of agar as shown in figure 1.

When these samples were divided into groups depending upon the average count upon the four kinds of agar the medium giving the highest counts for the different groups is indicated in table 4.

The average bacterial counts of samples tabulated in groups I, II and III when arranged according to their pH scores indicates that while the pH score does not check with the bacterial count in many cases, it does check in a general way. Samples scoring 75, 70, 65, 60, and 55, because of the small numbers

TABLE 4

Medium giving highest counts for the different groups

NUMBER OF BACTERIA PER CUBIC CENTIMETER AVERAGE WITH 4 KINDS OF AGAR	HIGHEST AVERAGE WITH	NEXT HIGHEST WITH	NEXT LOWEST WITH	LOWEST AVERAGE WITH
Under 10,000	Bacto agar	Agar A	Infusion agar	Extract agar
10,000– 25,000	Agar A	Extract agar	Bacto agar	Infusion agar
25,000– 50,000	Bacto agar	Agar A	Infusion agar	Extract agar
50,000– 100,000	Bacto agar	Agar A	Infusion agar	Extract agar
100,000– 200,000	Bacto agar	Agar A	Infusion agar	Extract agar
200,000–1,000,000	Agar A	Infusion agar	Bacto agar	Extract agar
Over 1,000,000	Bacto agar	Agar A.	Extract agar	Infusion agar

TABLE 5

Showing the relation of average bacteriological counts and average of pH scores

NUMBER OF SAMPLES	WITH A pH SCORE OF	AVERAGE BACTERIAL COUNT ON FOUR KINDS OF MEDIA
7	100	10,300
10	95	34,200
10	90	44,600
4	85	63,500
10	80	92,800
8	75, 70	150,000
13	65, 60, 55	562,000
14	50	5,086,000
13	45	4,178,000
2	40	2,714,000

of samples in the various groups, do not check when taken separately but do check when arranged together as shown in table 5.

The samples of milk scoring 50, 45 and 40 show a decrease in bacterial count. This is probably due to the fact that some of the inert types find the increased activity of the acid bacteria unfavorable.

TABLE 6

Comparisons of the pH score, reductase grade and the bacterial plate count on 82 samples of milk

LABORATORY NUMBER	pH SCORE	REDUCTASE	BACTERIA PER CUBIC CENTIMETER BACTO AGAR, pH 6.8	LABORATORY NUMBER	pH SCORE	REDUCTASE	BACTERIA PER CUBIC CENTIMETER BACTO AGAR, pH 6.8
1	90	Good	10,000	42	45	Fair	175,000,000
2	80	Good	55,000	43	55	Bad	65,000,000
3	80	Good	50,000	44	55	Very bad	2,000,000
4	95	Good	10,000	45	45	Bad	60,000,000
5	80	Good	120,000	46	35	Very bad	140,000,000
6	55	Fair	1,500,000	47	75	Fair	1,800,000
7	75	Fair	300,000	48	55	Bad	75,000,000
8	80	Good	160,000	49	45	Very bad	120,000,000
9	95	Good	85,000	50	45	Bad	110,000,000
10	90	Good	80,000	51	45	Bad	45,000,000
11	95	Good	110,000	52	45	Very bad	160,000,000
12	50	Good	3,000,000	53	45	Bad	80,000,000
13	95	Good	6,000	54	50	Bad	30,000,000
14	90	Good	20,000	55	35	Very bad	50,000,000
15	75	Fair	6,000	56	50	Fair	4,000,000
16	95	Good	100,000	57	45	Very bad	840,000
17	90	Good	25,000	58	55	Fair	35,000
18	80	Good	35,000	59	55	Fair	220,000
19	65	Good	40,000	60	55	Fair	420,000
20	95	Good	10,000	61	55	Fair	9,000,000
21	90	Good	24,000	62	50	Fair	84,000,000
22	85	Fair	220,000	63	55	Fair	42,000
23	80	Good	80,000	64	55	Fair	660,000
24	85	Good	30,000	65	55	Fair	460,000
25	75	Good	500,000	66	35	Very bad	84,000,000
26	80	Good	30,000	67	50	Fair	280,000
27	95	Good	16,000	68	50	Bad	1,200,000
28	50	Bad	65,000,000	69	55	Fair	30,000,000
29	40	Very bad	110,000,000	70	50	Bad	1,500,000
30	45	Very bad	50,000,000	71	45	Bad	700,000
31*				72	50	Bad	850,000
32	45	Very bad	160,000,000	73	90	Good	300,000
33	35	Very bad	175,000,000	74	50	Fair	100,000
34	40	Very bad	90,000,000	75	50	Fair	980,000
35	45	Very bad	125,000,000	76	45	Bad	2,000,000
36	45	Very bad	70,000,000	77	45	Bad	40,000,000
37	50	Very bad	45,000,000	78	50	Bad	2,500,000
38	50	Very bad	65,000,000	79	85	Fair	40,000
39	35	Very bad	180,000,000	80	55	Fair	700,000
40	55	Fair	5,000,000	81	55	Fair	500,000
41	55	Fair	6,000,000	82	55	Fair	250,000

*Plate contaminated.

This table does not include the nine samples of milk in group III which were incubated at 37°F. or the two samples of pasteurized milk in group I.

A matter, closely related to the above, is some comparisons which were made between the pH method, the reductase test, and the official plate count of bacteria in milk. In this work there are 82 samples of milk which were tested by the three methods. The plating technique was that given in the Fourth Edition of "Standard Methods of Milk Analysis," published by the American Public Health Association. The reductase test was that given as a provisional method in the same publication. Methylene blue tablets sold by the National Aniline and Chemical Company were used. Correction as to the strength of the methylene blue solution was noted.

The results are recorded in table 6.

A study of the data reveals the fact that the pH method correlates as well with the average bacterial count as does the reductase test. The following parallel comparisons will show this.

	Average bacteria Per centimeter
Reductase ratings:	
Good (24 samples)	204,000
Fair (24 samples)	6,104,000
Bad (15 samples)	38,583,000
Very bad (18 samples)	100,000,000
pH score ratings:	
Good	68,000
Fair	529,000
Bad	19,000,000
Very bad	90,000,000

Looking at the individual ratings the following facts are brought out:

2 samples classified as good by reductase test had excessively high counts
20 samples classified as fair by reductase test had excessively high counts
0 samples classified as poor by reductase test had low counts
0 samples classified as very poor by reductase test had low counts

2 samples classified as good by pH score had excessively high counts
3 samples classified as fair by pH score had excessively high counts
3 samples classified as poor by pH score had low counts
0 samples classified as very poor by pH score had low counts

SUMMARY

1. When plating methods are used, no one medium should be expected to give results which indicate correctly the condition of all grades of milk.

2. The pH score proved more efficient as a means of detecting slight changes in the history of milk than did the plating methods tried.

3. The methods as a whole were efficient in detecting slight changes in the history of the samples as follows:

In samples with bacterial counts under 25,000—45.8 per cent efficient.

In samples with bacterial counts between 25,000 and 100,000—71.4 per cent efficient.

In samples with bacterial counts between 100,000 and 1,000,000—85.0 per cent efficient.

In samples with bacterial counts over 1,000,000—97.0 per cent efficient.

It seems that the medium giving highest average counts depends upon the predominant groups present in the sample studied. This may account for divergent results obtained by various workers.

4. In 81 comparisons of the pH score with the reductase ratings and the bacterial plate counts, the pH score checked as well with the number of bacteria per cubic centimeter as did the reductase rating, although neither is an absolute measure of the bacterial content.

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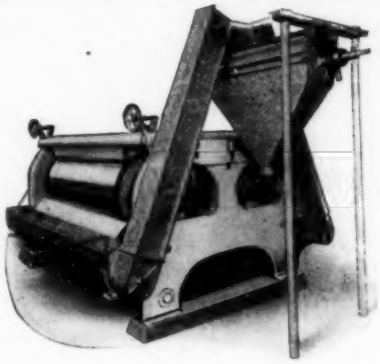

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